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Genetic Diversity, Genetic Variation and Identification of Quantitative Trait Loci (QTL) Associated with Biomass Yield and Establishment-Related Traits in Lowland Switchgrass (*Panicum virgatum* L.)

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To the Graduate Council:

I am submitting herewith a dissertation written by Cheryl Ontolan Dalid entitled "Genetic Diversity, Genetic Variation and Identification of Quantitative Trait Loci (QTL) Associated with Biomass Yield and Establishment-Related Traits in Lowland Switchgrass (*Panicum virgatum* L.)." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

Hem S. Bhandari, Major Professor

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DEDICATION

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"[4] He will wipe away every tear from their eyes, and death shall be no more, neither shall there be mourning nor crying nor pain any more, for the former things have passed away."

~Revelations 21:4

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ABSTRACT

Switchgrass is a warm-season C4 grass used for biofuel production. The primary goal of this study is biomass yield improvement for use as a bioenergy feedstock. The research plan was partitioned into three main objectives: (i) evaluate the genetic diversity among lowland switchgrass populations using microsatellite markers; (ii) assess genetic variation in an Alamo half-sib (AHS) population developed through phenotypic selection; (iii) and identify quantitative trait loci (QTL) associated with biomass yield and establishment related seed traits using a Nested Association Mapping (NAM) population. The genetic diversity study on lowland switchgrass showed significant phenotypic variations ($P<0.05$) among and within germplasm accessions. The Analysis of Molecular Variance (AMOVA) revealed that among genotypes within populations and among populations explained 84 and 16% of molecular genetic variations. The Principal Coordinate Analysis (PCoA) and cluster analysis separated switchgrass populations according to the ancestral background. The study on genetic variation showed significant variation ($P<0.05$) among AHS for biomass yield, tillering ability, and spring vigor, suggesting the importance of additive genetic variation in these traits. Results also showed great potential for biomass yield improvement through selection based on family performance. Using 10% selection intensity, parental control of two, and a narrow-sense heritability estimate of 0.11, gain per cycle selection from half-sib family selection is estimated to be 23%. The study on identification of QTLs associated with biomass yield and establishment related traits showed significant variation among NAM families and genotypes within families for biomass yield, seed weight, and germination ($P<0.05$). A total of 785 significant markers for biomass yield were detected ($P<0.05$) of which 176 were for seed weight and 131 for germination. Composite interval mapping revealed 21 QTLs for biomass yield across locations in 2 years the highest LOD score of 7.6 detected in chromosome Ia that explained

a 12.5% of the phenotypic variation. Two QTLs located in chromosome VIIIb for seed weight explained 5.2% and 4.6% phenotypic variation. One QTL for germination located in chromosome IXa explained 5.2% of the phenotypic variation. The results of these studies will be useful for future breeding efforts in switchgrass and other perennial grasses.

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INTRODUCTION AND LITERATURE REVIEW

SWITCHGRASS AS FEEDSTOCK FOR BIOFUEL PRODUCTION

In a world where climate change is a relevant concern and fossil fuels are limited in supply, studies regarding alternative sources of energy are vital. Biofuels such as ethanol and biodiesel are promising alternatives that have gained popularity over the years due to its renewability and positive environmental effect. According to the 2011 study by the United States Department of Energy, corn is the main feedstock used in bioethanol production. However, there are trade-offs in utilizing food crops, conversion of croplands dedicated to food production to bioenergy feedstock production could occur (Pimentel and Patzek, 2005). Thus, research was shifted to sources of biomass feedstock that can be sustainably produced under marginal environments.

As a result of the energy crisis that USA faced in 1973, the Department of Energy (DOE) and Oak Ridge National Laboratory (ORNL) initiated energy crop research to identify fast-growing trees and herbaceous plant species that could be potential sources of renewable energy (McLaughlin *et al*., 1999; Parrish *et al*., 2012; Wright and Turhollow, 2010). In 1991, after screening several herbaceous plant species, switchgrass was selected as the model herbaceous crop for bioenergy research (McLaughlin *et al*., 1999; Wright and Turhollow, 2010). With the introduction of the Energy Independence and Security Act (EISA) of 2007, more research on switchgrass has been established. The EISA mandates that by the year 2022, approximately 1.36×10^{11} L of fuel ethanol are to be produced in the US with 6.06×10^{10} L from lignocellulosic biomass (Qualls *et al*., 2012).

BIOENERGY FROM LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass consists of structural carbohydrates such as cellulose and hemicellulose, and lignin (Mitchell *et al*., 2014) derived from plant materials (McKendry, 2002). In biofuel production, high cellulose concentration and low lignin are desired (Chang and Holtzapple, 2000; Lee *et al*., 2007). Biomass can be converted to bioenergy through biochemical and thermochemical processes (Ni *et al*., 2006).

Most of the dry biomass components are composed of structural carbohydrates that be hydrolyzed to sugars and then converted to alcohols such as ethanol and methanol through the biochemical conversion process of fermentation (Mitchell *et al*., 2014; Wyman, 1994). Fuel from lignin, on the other hand, is not converted through fermentation. The lignin portion of the feedstock can be recovered for thermochemical energy conversions (Boateng *et al*., 2007) through gasification and pyrolysis (McKendry, 2002; Zhang *et al*., 2010) to produce syngas, and bio-oils (Zhang *et al*., 2010).

Cell wall composition of lignocellulosic crops determines the efficiency of biofuel production due to cell wall recalcitrance, or the resistance of cell wall to respond to chemical or enzymatic degradation to simple sugars (Himmel *et al*., 2007). While higher cellulose and hemicellulose contents directly contribute to the ethanol yield (Badger, 2002), lignin is considered a major factor affecting recalcitrance (Bajpai, 2016).

Lignin is part of the cell wall that aids in plant stability and protection from pathogens (Frei, 2013). Lignin is important for plant's structural stability and defense; however, it hinders enzyme access to cellulose and hemicellulose which are fermentable polysaccharides (Frei, 2013; Vanholme *et al*., 2010). Thus, overcoming cell wall recalcitrance should not sacrifice plant defense

systems and structural stability of cell walls. Transgenic methodologies have shown potential in developing switchgrass with high-quality feedstock composition by reducing lignin content by at most 12% (Baxter *et al*., 2014; Fu *et al*., 2011) and 14% (Baxter *et al*., 2014; Fu *et al*., 2011) without compromising biomass yield and disease susceptibility in field condition (Baxter *et al*., 2014; Li *et al*., 2017).

ENVIRONMENTAL BENEFITS

Aside from having a high biomass yield potential, switchgrass was chosen for biofuel production because of the expected positive environmental effects. Because soil tillage is only needed during the establishment year, there is reduced soil erosion (Ma *et al*., 2000). About 95% reduction in soil erosion in addition to a 90% reduction in pesticide use was observed when growing switchgrass in comparison to corn and soybean (Hohenstein and Wright, 1994). Bransby *et al*. (1998) demonstrated that switchgrass could improve carbon sequestration in farm soils. It has been reported that switchgrass can store 1.1 Mg of carbon hectare^{-1} annually (Gebhert *et al*., 1994). This could be attributed to the extensive deep root system of switchgrass, which accounts for almost 80% of the total biomass (Liebig *et al*., 2005).

A study by Entry and Watrud (1998) elucidated how switchgrass can be used in soil remediation. They used Alamo to remediate soil contaminated with cesium-137 and strontium-90. Such elements are products of nuclear testing and nuclear reactor accidents. Their results showed a 36% removal of cesium and a 44% removal of strontium in a span of five months. Switchgrass also improves wildlife and avian habitat since harvesting usually starts in the fall when the breeding season for grassland birds is almost over, thus causing minimal disturbance for nesting birds (Roth *et al*., 2005).

SWITCHGRASS GENETICS

Switchgrass (*Panicum virgatum* L.) is a warm-season, perennial, C4 grass used for forage, soil conservation, and biofuel production (Casler, 2012; McLaughlin and Walsh, 1998). It belongs to the Paniceae tribe in the subfamily Panicoideae of the Poaceae (Gramineae) family (Giussani *et al*., 2001). It is a cross-pollinated, polyploid species with a genome constitution that varies from diploid to dodecaploid with a basic chromosome number of 9 (Martinez-Reyna and Vogel, 2002). Switchgrass has a high gametophytic self-incompatibility due to an *S*–*Z* incompatibility system (Vogel, 2004). The mode of reproduction of switchgrass is both through seed production and vegetative propagation. It has a vigorous root system that can extend to 3.5 meters deep (Weaver, 1968) thus giving switchgrass an advantage in water and nutrient uptake (McLaughlin *et al*., 1999).

Switchgrass is adapted to a wide range of ecosystems in North America, ranging from Mexico to Canada (Stubbendieck *et al*., 1997). There are two ecotypes in switchgrass classification, the lowland and upland ecotypes. These ecotypes are associated with the different cytoplasm types, "L" and "U" respectively, which were determined through chloroplast DNA (cpDNA) polymorphisms (Hultquist *et al*., 1996; Vogel, 2004). There is a deletion of 49 nucleotides in the non-coding region of chloroplastic trnL DNA that is specific to lowland cytotypes (Missaoui *et al*., 2006). Lowland and upland ecotypes also differ in their morphological characteristics and adaptation (Bouton, 2007). Lowland ecotypes are adapted to the wetlands in the southern regions with mild winter temperature. Lowland switchgrass ecotypes are mostly tall, thick-stemmed with long and wide leaves, flower late in the season, and produce high biomass yield (Bouton, 2008; Vogel, 2004). Upland ecotypes are adapted to dry environments in the northern region where the temperature is relatively cold. Upland ecotypes have short plants, thin-stemmed, with fine leaves, have low biomass yields, but are tolerant to drought (Lowry *et al*.,

2014; Vogel, 2004). Lowland ecotypes are mostly tetraploids with 2n = 4x = 36 chromosomes while upland ecotypes are mostly octaploids with 2n = 8x = 72 chromosomes and few of them are tetraploids (Casler *et al*., 2011; Gunter *et al*., 1996; Wullschleger *et al*., 1996; Zhang *et al*., 2011).

Due to the outcrossing and polyploid nature of switchgrass, they have maintained high genetic diversity, with each population being highly heterogeneous and individual plants within each accession highly heterozygous (Parrish and Fike, 2005) which is useful for genetic improvement through breeding. The USDA National Plant Germplasm System (NPGS)-Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov/) maintains a collection of switchgrass germplasms. As of the third quarter of 2017, there were 167 available accessions in the GRIN germplasm repositories representing collections from 27 US states and three other countries (Argentina, Belgium, and Turkey). A study by Das *et al*. (2004) observed a high amount of genetic variation in biomass yield in three different populations of lowland switchgrass.

As an important species for bioenergy feedstock, understanding of genetic variation among and within different germplasm accessions of switchgrass will aid in their efficient utilization in cultivar development. Several studies have utilized molecular markers to assess the extent of genetic diversity and relatedness in switchgrass (Cortese *et al*., 2010; Gunter *et al*., 1996; Nageswara-Rao *et al*., 2014; Narasimhamoorthy *et al*., 2008; Zhang *et al*., 2016). Narasimhamoorthy *et al*. (2008) assessed genetic diversity in 31 switchgrass populations from USDA-GRIN that represented landraces from 20 US states. They assessed genetic diversity using expressed sequence tags-simple sequence repeats (EST-SSR) markers where each accession was represented by 6 genotypes. Separation of upland and lowland ecotypes with 9 potentially lowland populations clustering together were elucidated in their results. A study by Zalapa *et al*. (2011)

also included both upland and lowland switchgrass but utilized 2-16 genotypes per population in evaluating diversity using SSR and chloroplast markers. Their results showed discrimination of populations according to ecotype and geographical origin. Cortese *et al*. (2010) studied genetic diversity of 12 switchgrass populations from the Northeast US, which were mostly uplands. In their study, each population was represented by 48 genotypes and diversity was assessed using both morphological and EST-SSR marker data. Their results also differentiated populations based on geographical origin. The study by Nageswara-Rao *et al*. (2014) included both native landrace populations and cultivated varieties of lowland switchgrass collected from Central and East Tennessee. They determined their genetic diversity using SSR and chloroplast markers, and 12 genotypes per population. Their results showed a clear differentiation between native landrace and cultivated switchgrass populations with the native landrace switchgrass populations exhibiting higher mean genetic diversity compared to cultivated switchgrass populations.

CULTIVAR IMPROVEMENT

Genetic improvement through breeding of new varieties is the most effective and inexpensive way of increasing biomass yield (Bartley *et al*., 2013). Switchgrass is a relatively new species with respect to its cultivar breeding history. Most of the available cultivars are the results of direct selection from native germplasm and only a few cultivars released involved some systematic breeding (Casler, 2012). Early breeding for switchgrass using regional germplasms started at the University of Nebraska in the 1950s (Eberhart and Newell, 1959) but was limited to direct selection from germplasm collections (Vogel *et al*., 2010). Prior switchgrass breeding programs concentrated on improvement of biomass yield, *in vitro* dry matter digestibility (IVDMD), cell wall composition, plant height, tiller number, seed yield, protein concentration, rust resistance, maturity, and stress tolerance (Vogel and Jung, 2001; Vogel *et al*., 2010). After the

release of the first switchgrass cultivar, 'Nebraska 28', several germplasms have been evaluated and improved by exploiting its high genetic variability (Vogel *et al*., 2010). Following the research initiative for alternative source of renewable energy from plant biomass where switchgrass was chosen as a model herbaceous species for the bioenergy feedstock production in the United States, research interest in enhancing switchgrass biomass yield and feedstock quality became prominent.

Biomass yields of currently available cultivars are inadequate and its improvement is critically important for the profitability of switchgrass as a bioenergy crop (Perrin *et al*., 2008; Schmer *et al*., 2008). Currently, there are 15 switchgrass cultivars that resulted from selection and breeding which includes 9 lowland cultivars namely 'BoMaster', 'Cimarron', 'Colony', 'EG1101', 'EG1102', 'Expresso', 'Liberty', 'Performer', and 'TEM-LoDorm'. However, these varieties are not enough to meet the cultivar needs of diverse environments.

Switchgrass breeding methods include recurrent phenotypic selection with some restrictions (Burton, 1982) and genotypic selection based on half-sib or full-sib progeny-performance (Casler and Brummer, 2008). However, it has been observed that the rate of genetic gain in yield of perennial grasses is slow compared to the annual grain crops (Casler and Brummer, 2008; Humphreys, 2005). In bahiagrass, the recurrent restricted phenotypic selection resulted in a 2% to 6% gain in forage yield per cycle of selection (Burton (1982). Genotypic selection based on half-sib or full-sib family performance could improve selection gain (Brummer and Casler, 2009, Casler and Brummer, 2008; Vogel and Burson, 2004). However, conventional breeding efforts take longer time as switchgrass' first-year yield shows poor correlation with the plant performance in succeeding years, thus breeding populations require evaluating at least two years before making any selection. This long selection cycle limits the rapid genetic improvement, thus an efficient system that can overcome this challenge is warranted.

In developing cultivars with improved biomass yield, it is also advantageous to find traits that are correlated. Understanding the relationship between biomass yield and agronomic traits could aid breeders in the selection process. Bhandari *et al*. (2011) found a significant positive phenotypic correlation between plant height and stem thickness (r=0.77) and a significant positive phenotypic correlation of plant height (r = 0.56), stem thickness (r= 0.52), and tillering ability (r=0.45) with biomass yield. These relationships were consistent with a previous study conducted by Das *et al*. (2004). Das and Taliaferro (2009) also reported a positive phenotypic correlation between seed yield per plant and seed number per panicle in two locations (r = 0.76 at Chickasha and r = 0.72 at Perkins). Knowing these relationships would aid in breeding for switchgrass with enhanced biomass yield and adequate amount of seed for commercial distribution.

Conventional breeding has shown great potential in switchgrass cultivar improvement. However, the long selection cycles hinder rapid genetic improvement. Genomics research offers great opportunities that could accelerate switchgrass improvement for biofuel production. Molecular breeding is an interdisciplinary science that incorporates molecular marker technology and genomics into conventional breeding methodologies for crop improvement (Moose and Mumm, 2008). Molecular markers are DNA segments used to detect allelic variations with ease due to their stable presence in plants (Collard and Mackill, 2008; Singh *et al*., 1999; Zhang *et al*., 2016). Several studies on switchgrass genetics have utilized molecular markers to evaluate the extent of genetic diversity and relatedness (Cortese *et al*., 2010; Gunter *et al*., 1996; Nageswara-Rao *et al*., 2014; Narasimhamoorthy *et al*., 2008; Zhang *et al*., 2016). With this information, genetic variation among and within germplasms of switchgrass can be maintained for future allele-mining studies and/or for selection of parents for cultivar development.

Despite being a relatively new species in terms of breeding history, resources for genomics studies are now available for switchgrass. The first linkage map was developed based on a biparental mapping population consisting of 85 genotypes that were derived from a cross between an upland tetraploid, Summer, and a lowland tetraploid Alamo, AP13 (Missaoui *et al*., 2005). They utilized 102 restriction fragment length polymorphic (RFLP) markers and identified eight homology groups out of the expected nine in switchgrass. Serba *et al*. (2013) used the expanded biparental mapping population used by Missaoui *et al*. (2005) with 191 genotypes which included the original 85 genotypes to develop a linkage map using SSRs and diversity array technology (DArT) markers and a larger population (191 genotypes). Okada *et al*. (2010) reported the first complete linkage map of switchgrass using 238 F1 genotypes derived from a cross between Kanlow and Alamo. They utilized simple sequence repeats markers (SSR), expressed sequence tags-sequence tag sites (EST-STS), and EST-SSRs. Their result showed that switchgrass is an allotetraploid with disomic inheritance and 18 pairs of chromosomes.

Several QTL studies in different crop species have been successfully conducted in the past (Hackett *et al*., 2013; Li *et al*., 2008; Liu *et al*., 2006; Santra *et al*., 2008; Xu and Mackill, 1996). In the case switchgrass, Lowry *et al*. (2015) used a mapping population derived from Alamo × Kanlow genotypes and identified 27 significant QTLs, 2 of which are QTLs for biomass yield with 10% and 12% phenotypic variation explained which showed positive additive effects. Using a cross between lowland AP13 (Alamo genotype) and upland VS16 (Summer genotype), Serba *et al*. (2015) identified 34 QTLs related to biomass yield with 3.5 to 15.3% phenotypic variation explained and 38 QTLs related to plant height with 4.3 to 17.4% phenotypic variation explained which showed mostly negative additive effects. Chang *et al*. (2016) identified 20 QTLs for tillering-related traits with 5.7 to 17.8% phenotypic variation explained from a biparental cross

between a northern lowland genotype, NL 94, and a southern lowland genotype, SL 93. With the identification of molecular markers associated with the QTLs for important traits, switchgrass improvement could be hastened through marker-assisted breeding.

OBJECTIVES OF THE STUDY

At the University of Tennessee, Knoxville, switchgrass breeding is focused on biomass yield improvement for use as a bioenergy feedstock. The research plan for this dissertation is outlined in three main objectives:

a.) Evaluate the genetic diversity among lowland switchgrass populations using microsatellite markers;

b.) Assess genetic variation in the Alamo population developed through phenotypic selection;

c.) Identify QTLs associated with biomass yield and establishment related traits using a Nested Association Mapping (NAM) population.

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CHAPTER 1

GENETIC DIVERSITY AMONG NATIVE LANDRACES AND SELECTED POPULATIONS OF LOWLAND SWITCHGRASS

(A version of this chapter has been submitted to BioEnergy Research, authored by Cheryl O. Dalid, Arnold M. Saxton, Fred L. Allen, Vince Pantalone, Santosh Nayak, Malay Saha, and Hem S. Bhandari)

Cheryl O. Dalid conducted the experiments, collected and analyzed the data, and wrote the manuscript.

ABSTRACT

Understanding the extent of genetic variation among and within different germplasm accessions of switchgrass will aid to their efficient utilization in cultivar breeding. Twenty-two populations of lowland switchgrass including 11 Plant Introduction accessions and 11 improved populations were evaluated in the field at East Tennessee Research and Education Center (ETREC), Plant Science Unit, Knoxville. Each population was represented by 14 genotypes. Phenotypic data on biomass yield and important phenotypic traits were recorded. Molecular diversity in these germplasms was assessed using 14 genotypes per population and sixty sequence repeats (SSR) markers. In the molecular analysis, two upland populations 'Cave-in-rock' and 'Summer' were included for comparison. Results showed significant phenotypic variations ($P<0.05$) among and within germplasm accessions. Cluster analysis using phenotypic data revealed four groups, but the separation did not follow ancestral relationships. Molecular analysis revealed landraces exhibiting higher genetic variation, larger number of unique alleles (157), higher polymorphism (32%) while improved populations showed reduced diversity, less polymorphism (27%) and possess fewer unique alleles (52). The Analysis of Molecular Variance (AMOVA) revealed that among genotypes within populations and among populations explained 84 and 16% of molecular genetic variations. The Principal Coordinate Analysis (PCoA) and cluster

analysis separated switchgrass populations according to the ancestral background. Several accessions were between upland and lowland populations indicating their origin from upland × lowland hybridization. Reduced diversity in improved populations warrants that recurrent selection should consider a breeding approach that ensures adequate genetic diversity through the cycles of selection.

Abbreviations: SSR Simple Sequence Repeats, AMOVA Analysis of Molecular Variance, PCA Principal Component Analysis, PCR Polymerase Chain Reaction.

INTRODUCTION

As a result of the energy crisis in 1973, the United States Department of Energy (DOE) and Oak Ridge National Laboratory (ORNL) initiated energy crop research to identify potential sources of renewable energy from plant biomass (McLaughlin *et al*., 1999; Parrish and Fike, 2005; Wright and Turhollow, 2010). After screening several herbaceous plant species, switchgrass (*Panicum virgatum* L.) was selected as the model herbaceous crop for the development of lignocellulosic bioenergy feedstock (McLaughlin *et al*., 1999; Sanderson *et al*., 2006; Wright and Turhollow, 2010). Since then, there have been significant research efforts to optimize switchgrass for biomass feedstock production.

Switchgrass is a perennial rhizomatous grass native to the North American prairie lands. It is a warm-season, C4 grass, with high biomass production potential under low inputs environments. It belongs to the Paniceae tribe in the subfamily Panicoideae of the Poaceae (Gramineae) family (Giussani *et al*., 2001). Switchgrass can reproduce sexually through seed and asexually through rhizome extension. It is a cross-pollinated, polyploid species with a genome constitution that varies from diploid (2n=2x=18) to dodecaploid (2n=12x=108) (Burton, 1942; Nielsen, 1944). Cross-pollination in switchgrass is attributable to self-incompatibility which is similar to S-Z gametophytic self-incompatibility system observed in other grasses (Martinez-Reyna and Vogel, 2002; Vogel, 2004).

Phylogeny analyses have revealed a wide diversity in switchgrass germplasms that are broadly grouped into two ecotypes, upland and lowland (Cortese *et al*., 2010; Gunter *et al*., 1996; Hultquist *et al*., 1996; Missaoui *et al*., 2006; Narasimhamoorthy *et al*., 2008; Zalapa *et al*., 2011). A few intermediate types were also reported which could have resulted from ancient hybridization

and gene flow between the two ecotypes (Zhang *et al*., 2011a). The two ecotypes differ in genomic DNA contents, lowland ecotypes are tetraploids (2n=4x=36) and upland ecotypes are mostly octaploids (2n=8x=72) with a few exceptions such as 'Summer' which is a tetraploid upland (Gunter *et al*., 1996; Hopkins *et al*., 1996; Lu *et al*., 1998; McMillan and Weller, 1959; Riley and Vogel, 1982; Zhang *et al*., 2011b). The two ecotypes are also characterized by two associated cytotypes, 'L' for lowland and 'U' for upland (Hultquist *et al*., 1996). There was a deletion of 49 nucleotides in the non-coding region of chloroplastic trnL DNA that is specific to lowland cytotypes (Missaoui *et al*., 2006).

Lowland and Upland ecotypes also differ in their morphological characteristics and adaptation (Bouton, 2007). Lowland ecotypes are adapted to the southern regions where the environment is warmer and wetter. Most of the populations of lowland ecotypes are tall, thick-stemmed with longer and wider leaves, flower later in the season, and produce high biomass yield (Bouton, 2008; Vogel, 2004). Upland ecotypes are adapted to drier environments in the northern region where the temperature is relatively colder. Upland ecotypes have shorter plants, thin-stemmed, with finer leaves, have lower biomass yields, but are more tolerant to drought (Lowry *et al*., 2014; Vogel, 2004). Lowland switchgrass, due to its high biomass yield potential, is the ideal candidate for the development of lignocellulosic biomass feedstock.

As an important species for bioenergy feedstock, understanding of genetic variation among and within different germplasm accessions of switchgrass will aid in their efficient utilization in cultivar development. The USDA National Plant Germplasm System (NPGS)-Germplasm Resources Information Network (GRIN) (http://www.ars-grin.gov/) maintains a collection of switchgrass germplasms. As of the third quarter of 2017, there were 167 available accessions in the GRIN germplasm repositories representing collections from 27 US states and three other

countries (Argentina, Belgium, and Turkey). At the time the current study started, there were approximately 20 accessions of lowland ecotypes available in GRIN. Due to the outcrossing and polyploid nature of switchgrass, these accessions are expected to have maintained high genetic diversity, with each population being highly heterogeneous and individual plants within each accession highly heterozygous (Parrish and Fike, 2005).

Several studies have utilized molecular markers to assess the extent of genetic diversity and relatedness (Cortese *et al*., 2010; Gunter *et al*., 1996; Nageswara-Rao *et al*., 2014; Narasimhamoorthy *et al*., 2008; Zhang *et al*., 2016). Molecular markers are nucleotide variations in DNA segments that are used in genetic studies due to their stable presence in plants (Collard and Mackill, 2008; Singh *et al*., 1999; Zhang *et al*., 2016). Various molecular marker systems have been developed for use in genetic studies including restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), expressed sequence tags (ESTs), microsatellites or simple sequence repeats (SSRs), EST-SSR markers (Collard and Mackill, 2008; Kesawat and Das Kumar, 2009), and single nucleotide polymorphisms (SNP) (Evans *et al*., 2017; Lu *et al*., 2013). SSR markers are the most commonly used molecular markers due to their codominant nature, high polymorphism, reliability, and reproducibility (Morgante and Olivieri, 1993; Powell *et al*., 1996). Despite rapid advancements in genomics tools, such as high-throughput genotyping by sequencing and RNAseq, SSRs are still popular due to their co-dominant nature, high repeatability and simplicity for breeders' use (Kalia *et al*., 2010).

Molecular markers have been utilized in assessments of genetic diversity of several crop species including switchgrass (Cortese *et al*., 2010; Narasimhamoorthy *et al*., 2008; Zalapa *et al*., 2011). Narasimhamoorthy *et al*. (2008) assessed genetic diversity in 31 switchgrass populations representing 20 US states that were acquired from USDA-GRIN using EST-SSR markers where

each accession was represented by 6 genotypes. Their results showed separation of upland and lowland ecotypes with 9 potentially lowland populations clustering together. Zalapa *et al*. (2011) also included both upland and lowland switchgrass but utilized 2-16 genotypes per population in evaluating diversity with SSR and chloroplast markers. Their result elucidated discrimination according to ecotype and geographical origin. Cortese *et al*. (2010) studied genetic diversity of 12 switchgrass populations from the Northeast US, which were mostly uplands in which each population was represented by 48 genotypes. They used both phenotypic and EST-SSR marker data. Their results also differentiated populations based on geographical origin. Nageswara-Rao *et al*. (2014) included both native landrace populations and cultivated varieties of lowland switchgrass from Central and East Tennessee and determined their genetic diversity using SSR and chloroplast markers, and 12 genotypes per population. Their study found a clear differentiation between native landrace and cultivated switchgrass populations and that native landrace switchgrass populations have higher mean genetic diversity compared to cultivated switchgrass populations.

The current study is specific to lowland accessions and includes both native landrace accessions and improved populations. We have used 14 genotypes per accession and both agronomic and EST-SSR marker data for the assessment of diversity. The objectives of this study were to assess genetic diversity among and within native populations and improved varieties of lowland switchgrass using phenotypic traits and SSR markers and compare genetic diversity between improved populations and landraces and to identify potential polymorphism patterns related to geographic origins.

MATERIALS AND METHODS

Plant Materials

The description of germplasm accessions included in this study is summarized in Table 1.1. Initially, twenty lowland switchgrass plant introduction (PI) accessions were obtained from USDA Germplasm Resource Information Network (USDA-GRIN) in Fall 2012. Nine additional improved cultivars and advanced populations were also included. To break dormancy, seeds were treated using 100% household bleach for 15 minutes followed by rinsing twice with tap water (Bhandari *et al*., 2011). Seeds were germinated in petri dishes at room temperature. Seven of the 20 PI accessions did not germinate, thus only 13 plant introduction accessions were included. After two weeks, germinated seedlings were potted in 72-well flats filled with greenhouse soil mix and raised in the greenhouse (26º/15º C day/night 16 h light). For phenotyping, we used 15 seedlings per accession. Each genotype was clonally multiplied to produce three ramets, each ramet to represent a replicate in the experiment.

Field Experiment

The field nursery was established in the summer of 2013 at ETREC, Plant Science Unit, Knoxville (35°53'56.9"N 83°57'15.6"W) for the assessment of genetic variation in biomass yield and other important traits. The seedlings were transplanted on July 16, 2013, using a randomized complete block design with 3 blocks. Each block was composed of 345 plants, i.e., one ramet × 15 genotypes × 23 accessions. For this study, data from 14 genotypes and 22 accessions were used. The experiment was established as a spaced-plant nursery with a spacing of 1.2 m × 1.2 m. Fertilizer was not applied during the establishment year. In each spring of the post-establishment years, 60 kg N ha^{-1} was applied. Pre-emergence herbicides, Prowl H_2O (Pendimethalin, BASF

Corporation, Research Triangle Park, NC) at 3.31 L ha^{-1} and Dual II Magnum (Metolachlor, Syngenta, Crop Protection, Inc. Greensboro, NC) at 2.84 L ha^{-1} were applied during spring of each year. Post-emergence herbicide, 2,4-D at 2.37 L ha^{-1} with surfactant at 1.18 L ha^{-1} was applied 60 days after transplanting.

Phenotypic Data Collection

Biomass yield was recorded in the Fall of 2014, 2015, and 2016. Biomass was harvested using Sickle bar mower set at 15 cm high. Five tillers were sampled from each plant in one replication for the estimation of moisture content. Samples were dried in a batch oven (Wisconsin Oven Corporation, East Troy, WI, USA) for 48 hours at 49^{o}C. Sample fresh weight and dry weight were obtained, moisture content at harvest was determined, and was used to obtain the dry matter yield of the corresponding plant in each replication. Agronomic traits such as plant height, stem thickness, tillering ability, spring regrowth, heading, growth habit, and leaf angle were recorded from individual plants. Plant height was measured from the base of the plant to the tip of the panicle prior to harvest. Stem thickness was scored from 1 to 5, 1 being the thinnest stem and 5 as the thickest. Tillering ability was scored using a scale of 1 to 9, 1 being the least number of tillers and 9 with the highest number. Growth habit was also scored on a scale of 1 to 9, 1 being upright and 9 as spreading. Leaf angle was recorded in the fall of 2014 by estimating the angle between the leaf sheath and the blade of the flag leaf based on visual observation. Days to heading was recorded in the fall of 2014 by determining the day in the Julian calendar when 50% of the reproductive tillers have headed. Spring regrowth was also recorded based on the Julian calendar.

Genotypic Data Collection

The 22 lowland germplasms were assessed for molecular genetic diversity using SSR markers. Fourteen genotypes were assessed for each of the germplasms, except one for which only 5 genotypes were sampled. Upland cultivars 'Cave-in-Rock', an octaploid (seven genotypes), and 'Summer' a tetraploid (3 genotypes), were also included for comparison.

DNA extraction

Young leaves were collected from field-grown plants. Samples were then placed in the lyophilizer for 72 hours. Lyophilized tissues were subsequently ground to a fine powder in liquid nitrogen using mortar and pestle. Ground tissues were transferred to 2ml microfuge tubes for DNA extraction. Extraction buffer and lysis buffer stocks were prepared as described in the DNA extraction protocol used in Diversity Arrays Technology (DArT) (https://www.diversityarrays.com/files/DArT_DNA_isolation.pdf). These buffer stocks were then used to prepare a fresh buffer solution. DNA extraction protocol used was also based on DArT. One milliliter of fresh buffer solution preheated to 65ºC was added to 2ml microfuge tubes with ground leaf tissues and incubated at 65ºC for 1 hour. Microfuge tubes were inverted every 20 minutes. After incubation, samples were cooled down for 5 minutes. One milliliter chloroform-isoamyl alcohol (24:1) was then added to the samples followed by vortexing for 30 minutes. Samples were centrifuged at room temperature for 20 minutes at 10,000 x g. The aqueous phase was transferred to a new microfuge tube and an equal amount (v/v) of ice-cold absolute isopropanol was added. Tubes were inverted 10 times until DNA was visible. Samples were again centrifuged at room temperature for 30 minutes at 10,000 x g and supernatant were discarded and washed two times with 2ml 70% ethanol. Dried DNA pellets were dissolved in 1x TE buffer and

DNA concentration was assessed using a Nanodrop spectrophotometer ND-1000 (Nanodrop Technologies, DE, USA). The DNA was then diluted to a DNA stock of 100ng/μl. Samples were then transported to the Noble Research Institute, LLC, for PCR analysis and genotyping.

Polymerase Chain Reaction (PCR) and Genotyping

A total of 103 genomic SSR markers developed for switchgrass at the Noble Research Institute were pre-screened by genotyping six random DNA samples of different switchgrass accessions. Sixty SSR markers showing polymorphism in the prescreening were selected for the assessment of genetic diversity (Serba *et al*., 2013). All PCR were prepared with a volume of 10µl containing 10ng DNA template, 5x colorless GoTaq® reaction buffer (Promega, California, USA), 2.0mM dNTPs, 10µM each of the reverse and M13 universal primer, 5µM of the forward primer, and 0.5U GoTaq® polymerase (Promega). The M13 universal primer used was labeled with fluorescent tags of either blue (FAM), green (VIC), yellow (NED), or red (PET). We followed touch down PCR using 384-well thermal cyclers. Four different PCR products, 3µl each, with different fluorescent labels were pooled with 10µl standard solution that contains deionized formamide and GeneScan-500LIZ size standard. Samples were then genotyped using an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) at the Plant Biology Department of the Noble Research Institute. All genotypes were visualized, manually checked, cleaned, and scored using the GeneMapper 3.7 software. Base pair scores were converted to binary scores with the presence of a PCR product scored as 1 and 0 for its absence. Although this method was developed for diploid, it has worked as well for lowland switchgrass which despite being tetraploid, shows disomic inheritance (Okada *et al*., 2010; Okada *et al*., 2011).

Data Analysis

Analysis of Phenotypic Data

Data on biomass yield and other agronomic traits were analyzed using GLIMMix in SAS (SAS Institute, 2013). Year was used as fixed, and blocks, accessions, and genotypes within accessions were used as random factors in the model. Genetic variation among accessions was estimated using single plant data recorded for 2014, 2015, and 2016. Least squares means for years were obtained and their statistical differences were determined based on Fisher's protected least significant difference ($P \leq 0.05$).

The means for biomass yield and other agronomic traits from the 22 switchgrass accessions were then subjected to principal component analysis (PCA) using NCSS (NCSS 10 Statistical Software, 2015). Two important principal components produced were used as input variables for cluster analysis using Ward's minimum variance method (Ward, 1963). Ward's method is one of the most common hierarchical clustering procedures used in the analysis of plant germplasm resources (Missaoui *et al*., 2006).

Analysis of Genotypic Data

Analysis of molecular variance (AMOVA) was done using GenAlEx 6.5 based on 9,999 permutations to allow partitioning of molecular variance into within and among germplasms. Analysis of Molecular Variance also allows the test of significance of partitioned components of variation through permutation tests. The ΦPT value is computed in AMOVA, which is similar to F statistics. This value tests the statistical differences among accessions (Excoffier *et al*., 1992).

Within-population diversity was compared between landrace populations and improved cultivars using change in allele frequency.

A genetic distance matrix among 22 accessions for binary data was generated from the presence (1) or absence (0) data of the 309 genotypes using GenAlEx Version 6.5 (Peakall and Smouse, 2012) based on the method by Huff *et al*. (1993). This distance matrix was then used for cluster analysis. Ward's minimum variance method was used to generate a dendrogram using the NCSS 10 Statistical Software (NCSS 10 Statistical Software, 2015).

Combined Analysis

Twenty-two accessions for which phenotypic data were available were included in the combined analysis. Means of phenotypic data were subjected to a PCA (Principal Component Analysis) using NCSS (NCSS 10 Statistical Software, 2015). Two important principal components that explained 99% of the phenotypic variation were generated from this analysis. For the genotypic data, the genetic distance matrix generated from the presence or absence of alleles data using GenAlEx Version 6.5 (Peakall and Smouse, 2012) was subjected to PCA using NCSS (NCSS 10 Statistical Software, 2015). Five important principal components that explained 90% of the genotypic variation were generated from the analysis. The 2 principal components from the phenotypic data and the 5 principal components from the genotypic data were used as input for cluster analysis using Ward's minimum variance in NCSS (NCSS 10 Statistical Software, 2015). Ward's minimum variance method makes use of ANOVA-based metrics in evaluating distances between groups or clusters and minimizes the pooled within-group sum of squares (SS) (Ward, 1963).

RESULTS

Variation in Biomass Yield and Agronomic Traits

Analysis of variance showed that 22 switchgrass populations differed in their mean biomass yields across years (2014, 2015, and 2016) ($P<0.01$; Table 1.2). Genotypes within accessions also differed in their mean biomass yields ($P<0.01$). It is noteworthy that among accession variation was greater than within accession variation. However, the 6-fold difference in mean biomass yields among accessions observed under space-plant evaluation may not necessarily hold true when accessions are evaluated in a competitive environment.

The 22 switchgrass accessions also differed in heading, plant height, tillering ability, stem thickness ($P<0.01$), and spring regrowth ($P<0.05$; Table 1.2). Switchgrass accessions were not different in growth habit and leaf angle. Genotypes within accession varied widely for plant height, tiller number, stem thickness, and spring regrowth ($P< 0.01$; Table 1.2). Biomass yield, plant height, stem thickness, and spring regrowth exhibited higher among accession variation than within accession variation. Such results could reflect the differences in frequencies of favorable genes attributable to different improvement status. Tillering ability and leaf angle exhibited higher within accession variation than among accession variation suggesting the influence of dominant genes in these traits. The effect of year was significant (Table 1.2; $P<0.001$) for biomass yield, tillering ability, plant height, stem thickness, growth habit, and spring regrowth. In terms of biomass yield, year 1 and year 2 yielded 25% and 71% of year 3 biomass production. However, this is not the case with plant height where year 1 already produced 88% of year 2 production.

The mean single plant biomass yield ranged from 0.44 to 3.32 kg. Alamo-HighGermP and Rambo produced the highest biomass yield (3.32 and 3.19 kg plant^{-1} respectively; Table 1.3) while

PI 414067 and PI 479292 produced the least amount of biomass (0.44 and 0.59 kg plant^{-1} respectively). Mean plant height varied widely and ranged from 158 to 272 cm with PI 414067 being the shortest and Rambo as the tallest. Alamo-HighGermP was the second tallest accession with 263 cm. Rambo and Alamo-HighGermP showed a 10% and 7% increase in plant height as compared to the Alamo-2 population. The mean tillering ability score ranged from 4 to 7 with PI 479292 having the lowest tillering ability score while Alamo-2, Cimarron, EG1101, Kanlow, Alamo-HighGermP, Alamo-HiSWP, and Alamo-HighGermS having shown superior tillering ability. The mean stem thickness score ranged from 1 to 4. PI 315727 and PI 414067 showed the lowest stem thickness score while Rambo and Alamo-HighGermP had the highest stem thickness score. Similar to plant height, Rambo and Alamo-HighGermP showed thicker stems as compared to Alamo population. It should be noted that Alamo-HighGermP and Rambo populations were derived from a single cycle of phenotypic selection based on plant vigor at maturity, and phenotypic selection seems to have improved biomass yield and yield components.

Switchgrass accessions also differ in growth duration and winter dormancy. The mean heading days ranged from 186 to 196 days with PI 476290 heading the earliest while PI 422003 heading the latest (Table 1.3). In terms of spring regrowth, the values ranged from 76 to 83 days (in Julian calendar). PI 422006, Alamo-2, Cimarron, EG1101, Alamo-HiSWP, and Alamo-HighGermS were the earliest and PI 414067and PI 479292 were the latest in spring green-up.

Cluster Analysis

Ward's analysis of the phenotypic data resulted in four groups (Figure 1.1) but clustering did not strictly follow ancestral or geographical origins. In this analysis, the accessions PI 414067 and PI 315727 from North Carolina and PI 479292 from Arkansas formed a distinct cluster. Further

review of literatures revealed that PI 414067 and PI 476292 were earlier classified as upland populations based on analysis of the non-coding region of chloroplast trnL gene (Missaoui *et al*., 2006; Zhang *et al*., 2016). The second group comprised mostly of Alamo and most of the Alamo-derived cultivars and experimental populations (Alamo-HighGermP, Rambo, Alamo-HiSWP, Alamo-HighGermS, and Cimarron). The third group is made up of accessions Performer28 and PI 476290 from North Carolina, and PI 422016 and PI 421901 from Florida. In the fourth group, Kanlow and Kanlow-HighGermS were grouped together with EG1101, an Alamo derived population and PI 422006, the GRIN accession of Alamo. The fourth group, which is slightly differentiated from the third group, includes PI 414065 and PI 421999 from Arkansas, TEM-LoDorm from Texas, and PI 315723 from North Carolina. In general, the germplasm populations showed minimum phenotypic diversity (Figure 1.1). It was interesting to see that commercial source of Alamo and Alamo-derived population including Cimarron (second group) separated from EG1101 Alamo accession from USDA-GRIN source, and EG1101, an Alamo derived population (group 4). This indicates that clustering based on phenotypic data may not be adequate to separate accessions based on genetic background.

Molecular Variation among Germplasm Accessions

Twenty-four switchgrass accessions including two uplands populations, Summer and Cave-in-rock, were analyzed using 60 SSR primer pairs that generated a total of 1,780 alleles (Table 1.4). For the accessions that were represented by 14 genotypes, the number of alleles per accession ranged from 444 to 686 where TEM-LoDorm, a cultivar from North Carolina, amplified the least number of alleles (444) and PI 479292, a landrace from Arkansas, amplified the most number of alleles (686) (Table 1.4). We also observed fewer alleles (272) for Alamo-HiSWP, a breeding line from Tennessee, but this could be due to having fewer plants (n=5) sampled for this

assay. Overall, 230 accession-specific alleles were detected with the highest number of unique alleles per accession from PI 422003 and PI 414067 (31, 36 respectively) and the lowest number of unique alleles per accession from Alamo-HiSWP, TEM-LoDorm, and Kanlow-HighGermS (0, 2, 2 respectively). The percentage of polymorphic loci within each accession ranged from 15% to 39% with an average of 29%. PI 479292 had the highest percentage of polymorphic loci. Alamo-HiSWP was observed to have the lowest percentage of polymorphic loci. Again, this may reflect the fact that this population was represented by only 5 genotypes as opposed to 14 genotypes sampled for the rest of the populations. When this population was excluded, mean polymorphism observed was comparable among landraces, experimental varieties, and the Alamo base population.

In grouping the accessions according to their present improvement status, 11 landrace accessions produced a total of 157 unique alleles while 11 improved cultivars and advanced populations produced only 52 unique alleles (Table 1.4). The overall percentage of polymorphic loci for landrace accessions was 32% while the improved cultivars and populations had 27% polymorphic loci (Table 1.4). When Alamo-HiSWP is excluded, improved cultivars and advanced lines will have 52 unique alleles and 28% polymorphism, which is not drastically different from the original result. These results showed reduced polymorphism and number of unique alleles in improved cultivars and advanced breeding populations.

Partitioning of Molecular Variance

The Analysis of Molecular Variance (AMOVA) revealed that most of the variation or genetic diversity occurred within accessions (84%) while variation or genetic diversity among accessions contributed 16% (Table 1.5). This result is comparable to earlier studies of

Narasimhamoorthy *et al*. (2008) and Cortese *et al*. (2010) where within accession variation is higher than among accessions. Results from 9,999 permutations suggest the significance of the overall ΦPT value (0.157, P<0.001) thus, revealing significant variation among accessions. When landrace accessions and improved/advanced were analyzed separately, we observed a reduction in molecular genetic diversity, both among and within populations, of improved varieties compared to landrace populations (Table 1.5). The smaller total molecular variation estimated for improved varieties as compared to landraces may reflect the fact that the improved varieties in this study were derived by polycrossing fewer selected genotypes.

The Principal Coordinate Analysis showed that two principal coordinates jointly explained 52% of the total genetic variation (Figure 1.2). All the Alamo and Alamo derived accessions formed a separate group (i.e., the lower left quadrant in Figure 1.2). Kanlow and Kanlow derived population, Kanlow-HighGermS and two accessions from Arkansas, PI414045 and PI 421999 formed a separate group as seen in the upper left quadrant of Figure 1.2. Somewhat distantly located in the same quadrant is Performer28, which is also derived from Kanlow. However, repeated cycles of selection and use of fewer clones used to produce this population may have differentiated this population from the rest of the Kanlow and Kanlow-derived populations. The third group formed around the center of the coordinates includes PI 476292, an accession from Arkansas, two accessions from Florida (PI 421901 and PI 422016), 3 accessions from North Carolina (PI 315723, PI 315727, and PI 476290), and PI 422003 from Texas. Results also showed the two upland ecotypes, summer and cave-in-rock and PI 414067 clearly separated from the rest of the populations (lower right quadrant in Figure 1.2).

Cluster analysis using Ward's minimum variance also produced four distinct clusters (Figure 1.3) that corresponded results from PCoA (Figure 1.2). Further analysis using Ward's

minimum variance method and combined phenotypic and molecular data (Figure 1.4) also resulted into four groups that were identical to results from PCoA (Figure 3) and Ward's cluster analysis using molecular data (Figure 1.4).

DISCUSSION

Lowland switchgrass populations differed greatly with respect to important phenotypic traits as well as genotypic markers (Table 1.2; Table 1.5). The analysis of variation using phenotypic data showed higher variation among accessions compared to variation among genotypes within accessions (Table 1.2). This could simply reflect the inclusion of both native landraces and improved cultivar and populations in the current analysis. The improved cultivars and population have been selected for biomass yield and yield components. In contrast, analysis of molecular variation demonstrated that a large amount of molecular variation (84%) is attributable to variation among genotypes within the populations, while only 16% of molecular variation is explained by the differences among accessions (Table 1.5). Our results showed that the genotypes within each improved population are less variable, had fewer unique alleles, and were less polymorphic compared to native unimproved germplasms (Table 1.3, 2.4, 2.5). Most of the improved populations used in this study have resulted from the single cycle of selection. If these populations were to go through repeated cycles of recurrent selection, the reduced diversity may become an issue limiting the scope for cultivar breeding.

Cluster analysis using phenotypic data clearly separated PI 414067, PI476292, PI315727 accessions from the rest of the lowland populations. Further review of literature revealed that PI414067 and PI476292 were classified as upland based on analysis of chloroplast trnL introns. Our study also showed PI414067 together with upland populations (Figure 1.2) further confirming this population could have been originally mischaracterized. PI 476292, despite being characterized as upland based on mutations on chloroplastic trnL introns, appeared in the center of the PCoA plot halfway between the known upland and lowland populations. In both cluster analysis using phenotypic data and using SSR markers, PI 315727 from North Carolina grouped

together with PI414067 and PI476292. However, the principal coordinate analysis showed this accession grouping with PI 476292 and five other accessions, two other accessions, PI 476290, PI 315723 from North Carolina, two accessions from Florida, PI 422016, and PI421901 and one accession, PI 422003 from Texas. These seven accessions could have resulted from upland x lowland hybridization. Zhang *et al*. (2011a) observed some genotypes of PI 422003 'PMT-785' as upland as shown by chloroplast DNA, but their morphology resembled lowland types.

Several diversity studies have used less than 14 plant samples per population (Nageswara-Rao *et al*., 2014; Narasimhamoorthy *et al*., 2008; Zalapa *et al*., 2011) while Cortese *et al*. (2010) used more than 14 genotypes. To address whether the number of genotypes used in our study was adequate, a subset of random samples of 5 and 10 genotypes were used to determine changes in polymorphism within each population (Table 1.6). Results showed an increase in sample size resulted in significant increase in recovery of polymorphic loci within each population (Table 1.6; Figure 1.5). There was an 8% increase in polymorphism by increasing sample size from 5 to 10. Another 4% increase in polymorphism was observed from using 14 genotypes instead of 10. It is also evident that as the sample size increased, more alleles were detected in each population (Table 1.6; Figure 1.5). Cluster analysis using Ward's method further confirmed that the 5 and 10 genotype samples produce clusters that did not follow ancestral relationship (Figure 1.6a, 2.6b). Thus, 5 and 10 genotypes may not be adequate to capture the amount of polymorphism needed to reasonable discrimination between accessions.

The common practice in recurrent selection based on half-sib or full-sib performance is to recombine 8-10 parental clones of superior performing families to produce improved cultivars. Our results showed a reduced level of molecular diversity in improved cultivars which could be an issue if the improved cultivar is to be used in recurrent selection. The issue could be addressed

by following among and within family selection that would ensure greater allelic diversity through the cycles of recurrent selections (Casler and Brummer, 2008). However, environmental influence on single plant performance could limit the genetic gain following AWF selection. Using at least two clonal replicates in family evaluation would allow parsing out residual variance thus improving the selection efficiency. In each cycle, 50 to 200 (i.e., depends on the number of families evaluated, and proportion selected) can be used to recombine into the improved population which would ensure greater diversity in the breeding population.

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APPENDIX 1

CHAPTER 1 TABLES AND FIGURES

Table 1.1. Description of lowland switchgrass populations used in diversity analysis.

Accession	Origin	Status	Base population	Description
Lowland				
PI 315723	North Carolina	Landrace		BN-8358-62
PI 315727	North Carolina	Landrace		BN-11357-63
PI 414065	Arkansas	Landrace		BN-14668-65
PI 421901	Florida	Landrace		Miami
PI 421999	Arkansas	Landrace		AM-314/MS-155
PI 422003	Texas	Landrace		PMT-785
PI 422006	Texas	Landrace		GRIN accession of Alamo
PI 422016	Florida	Landrace		PI 422016
PI 476290	North Carolina	Landrace		T 2086
PI 476292	Arkansas	Landrace		T 2100
PI 414067	North Carolina	Landrace		BN-8624-67
TEM-LoDorm	Texas	Improved Cultivar	Alamo	Cultivar derived from Alamo population after 4- cycles of selections; initial 3 cycles based on early germination of individual seed and cycle-4 selection based on early germination of seed progeny. Twenty-four clones were selected and recombined to produce TEM-LoDorm
EG1101	Georgia	Improved Cultivar	Alamo	Improved variety derived from Alamo; derived by intercrossing 25 genotypes which originated from five clones that were selected based on half-sib progeny performance
Alamo-2	Tennessee	Random Seed Increase	Alamo	Commercial cultivar grown in Tennessee
Cimarron	Oklahoma	Improved Cultivar	Alamo	Cultivar developed by intercrossing seven clones, six clones were selected from a population containing equal proportion of Alamo and PMT (Wu and Taliaferro, 2009) and one genotype included was presumably lowland contaminant in upland population.
Kanlow	Tennessee	Random Seed Increase	Kanlow	Commercial cultivar grown in Tennessee

Table 1.1 (continued).

Accession	Origin	Status	Base population	Description
Performer28	North Carolina	Improved Cultivar	Kanlow	Advanced population received from North Carolina State University; derived by 4 cycles of selection from population containing 11 lowland germplasms including Kanlow. It was derived from 8 clones that were selected for superior yield and digestibility (Burns *et al*., 2008).
Rambo	Tennessee	Improved Cultivar	Alamo	Experimental variety developed by intercrossing 10 clones selected from growers' field in east Tennessee based on phenotypic vigor at maturity.
Alamo-HighGermP	Tennessee	Advanced lines	Alamo	Experimental variety derived from intercrossing nine clones selected based on high germination of seeds (Bhandari, 2012; unpublished records)
Alamo-HiSWP	Tennessee	Advanced lines	Alamo	Experimental variety derived from intercrossing seven clones selected based on high seed weight these clones produced (Bhandari, 2012; unpublished records).
Alamo-HighGermS	Tennessee	Advanced lines	Alamo	Experimental variety derived from intercrossing earliest germinating seeds from nine clones selected for high germination; six seedlings per clones were included in crossing block (Bhandari, 2012; unpublished records).
Kanlow-HighGermS	Tennessee	Advanced lines	Kanlow	Experimental variety derived from intercrossing earliest germinating seeds from seven clones selected for high germination, six seedlings per clones included in crossing block (Bhandari, 2012; unpublished records).
Upland				
Cave-in-Rock	Illinois	Random Seed Increase	Cave-in-Rock	Cave-in-Rock was released by the USDA Natural Resources Conservation Service and the Missouri Agricultural Experiment Station; selected from a native stand in Illinois based on seedling vigor, disease resistance, higher seed yields, and resistance to lodging.
Summer	Nebraska	Random Seed Increase	Summer	Summer was released by the South Dakota Agricultural Experiment Station; a native collection from Nebraska and improved through mass selection for earliness, leafiness, and rust resistance.

Table 1.2. Component of variation of the 22 lowland switchgrass accessions for biomass yield and other agronomic traits from 2014-2016.

Source of Variation	Biomass Yield (kg plant^{-1})	Heading (days)[†]	Plant Height (cm)	Tillering Ability[‡]	Stem Thickness[§]	Spring Regrowth[¶] (days)	Growth Habit[#]	Leaf Angle[††]
				Variance Estimates				
Block	0.00	0.00	26	0.07	0.01	0.03	0.03	3
Accession	0.67**	5.82**	877**	0.68**	0.49***	3.69*	0.00	1
Plants(Accession)	0.20***	1.58	295***	1.09***	0.22***	1.22***	0.00	6
Block x Plants(Accession)	0.12***	47.90***	99	0.53***	0.07***	0.65**	0.00	112***
Year x Accession	0.32***	-	43*	0.03*	0.00	4.29***	0.55***	-
Year x Plants(Accession)	0.26***	-	32	0.05	0.02*	1.31***	1.01***	-
Residual	0.26***	0.98	1436***	1.07***	0.27***	5.24***	3.13***	1
				Test of fixed effects (*F* values)				
Year	68.45***	-	113***	84.54***	15.90***	228.99***	40.73***	-

[†] Days to heading = Julian calendar
[‡] Tillering ability = score 1 (<10) to 9 (>80)
[§] Stem thickness = score 1 (thinnest) to 5 (thickest)
[¶] Spring regrowth = Julian calendar
[#] Growth habit = score 1 (upright) to 9 (sprawling)
[††] Leaf angle = º angle from leaf sheath
* Significant at P<0.05.
** Significant at P<0.01.
*** Significant at P<0.001.

Table 1.3. Mean biomass yield and other agronomic traits of the 22 switchgrass accessions from 2014-2016.

Accessions	Biomass Yield (kg plant^{-1})	Heading (days)[†]	Plant Height (cm)	Tillering Ability[‡]	Stem Thickness[§]	Spring Regrowth[¶] (days)	Growth Habit[#]	Leaf Angle[††]
PI 315723	1.14	190	219	5	2	82	4	31
PI 315727	0.68	187	168	6	1	82	5	33
PI 414065	1.34	189	213	5	3	80	5	30
PI 414067	0.44	189	158	5	1	83	6	33
PI 421901	1.48	193	202	6	3	80	5	30
PI 421999	1.56	190	219	6	3	80	5	30
PI 422003	1.22	196	212	6	2	79	5	32
PI 422006	2.18	193	228	6	3	76	5	35
PI 422016	1.14	191	203	5	3	81	4	31
PI 476290	0.96	186	201	5	3	82	4	31
PI 476292	0.59	189	166	4	2	83	5	33
TEM-LoDorm[‡‡]	1.23	193	219	6	3	77	5	31
Alamo-2	2.98	194	244	7	3	76	5	31
Cimarron	2.51	194	253	7	3	76	5	32
EG1101	2.04	193	228	7	3	76	5	33
Kanlow	2.25	193	238	7	3	80	5	38
Performer28	0.96	191	199	6	2	80	5	30
Rambo	3.19	195	272	6	4	77	5	31
Alamo HighGermP[§§]	3.32	194	263	7	4	76	5	33
Kanlow HighGermS[¶¶]	2.17	193	233	6	3	80	5	36
Alamo HiSWP[##]	2.71	194	251	7	3	76	5	31
Alamo HighGermS[†††]	2.96	193	251	7	4	76	6	32
LSD	0.23**	3.19**	12**	0.5**	0.24***	0.84*	0.63	4.45

[†] Days to heading = Julian calendar
[‡] Tillering ability = score 1 (<10) to 9 (>80)
[§] Stem thickness = score 1 (thinnest) to 5 (thickest)
[¶] Spring regrowth = Julian calendar
[#] Growth habit = score 1 (upright) to 9 (sprawling)
[††] Leaf angle = º angle from leaf sheath
[‡‡] Temperature-Low Dormancy (Details in Table 1.1)
[§§] Alamo-High Germination Parents (Details in Table 1.1)
[¶¶] Kanlow-High Germination Seeds (Details in Table 1.1)
[##] Alamo-High Seed Weight Parents (Details in Table 1.1)
[†††] Alamo-High Germination Seeds (Details in Table 1.1)
* Significant at P<0.05.
** Significant at P<0.01.
*** Significant at P<0.001.

Table 1.4. Genetic diversity parameters of 24 switchgrass accessions.

Sample Name	Ecotype	Size	No. of Alleles	No. of Unique Alleles	% of Polymorphic Loci
PI 315723	L	14	530	4	30
PI 315727	L	14	593	12	33
PI 414065	L	14	490	7	27
PI 414067	L	14	622	36	35
PI 421901	L	14	621	13	35
PI 421999	L	14	552	8	31
PI 422003	L	14	583	31	33
PI 422006	L	14	653	10	37
PI 422016	L	14	469	10	26
PI 476290	L	14	490	7	28
PI 476292	L	14	686	19	39
TEM-LoDorm[†]	L	14	444	2	25
Alamo-2	L	14	603	6	34
Cimarron	L	14	471	9	26
EG1101	L	14	574	5	32
Kanlow	L	14	563	7	32
Performer28	L	14	365	5	20
Rambo	L	14	552	6	31
Alamo-HighGermP[‡]	L	14	540	5	30
Kanlow-HighGermS[§]	L	14	540	2	30
Alamo-HiSWP[¶]	L	5	272	0	15
Alamo-HighGermS[#]	L	14	566	5	32
Cave-In-Rock	U	3	288	6	16
Summer	U	7	442	15	25
					Mean
Lowland			1757	209	30
Upland			542	21	20
Landrace			1654	157	32
Improved			1323	52	27
Overall no. of alleles			1780	230	

[†] Temperature-Low Dormancy (Details in Table 1.1)
[‡] Alamo-High Germination Parents (Details in Table 1.1)
[§] Kanlow-High Germination Seeds (Details in Table 1.1)
[¶] Alamo-High Seed Weight Parents (Details in Table 1.1)
[#] Alamo-High Germination Seeds (Details in Table 1.1)

Table 1.5. Analysis of molecular variance (AMOVA) for 309 individuals from 24 switchgrass accessions based on 55 SSR markers.

Source	df	SS	MS	Estimate	% Variation
All[†]					
Among Accessions	23	6324	274	15	16
Within Accessions	285	23075	81	81	84
Total	308	29400		96	100
$\Phi PT = 0.157^{***}$					
Landrace					
Among Accessions	10	2909	291	15	15
Within Accessions	143	11810	83	83	85
Total	153	14719		97	100
$\Phi PT = 0.153^{***}$					
Improved/Advanced lines					
Among Accessions	10	2307	231	11	13
Within Accessions	134	10543	79	79	87
Total	144	12850		90	100
$\Phi PT = 0.128^{***}$					

[†]Includes uplands Summer and CIR
***Significant at P<0.0001 based on 9999 permutations
df = degree of freedom, SS = sum of squares, MS = mean squares, Estimate. = estimate of variance, % variation = percentage of total variation

Table 1.6. Number of alleles and percent polymorphism using three different sample sizes.

Sample Name	14 genotypes		10 genotypes		5 genotypes	
	No. of Alleles	% of Polymorphic Loci	No. of Alleles	% of Polymorphic Loci	No. of Alleles	% of Polymorphic Loci
PI 315723	530	30	467	26	316	18
PI 315727	593	33	510	29	346	19
PI 414065	490	27	436	24	314	17
PI 414067	622	35	565	31	405	22
PI 421901	621	35	521	29	384	21
PI 421999	552	31	493	28	343	19
PI 422003	583	33	517	29	400	22
PI 422006	653	37	543	30	374	21
PI 422016	469	26	408	23	300	17
PI 476290	490	28	402	23	310	17
PI 476292	686	39	595	33	415	23
TEM-LoDorm[†]	444	25	387	22	293	16
Alamo-2	603	34	512	29	370	21
Cimarron	471	26	409	23	301	16
EG1101	574	32	511	29	351	19
Kanlow	563	32	486	27	350	19
Performer28	365	20	328	18	251	14
Rambo	552	31	506	28	340	19
Alamo-HighGermP[‡]	540	30	486	27	359	20
Kanlow-HighGermS[§]	540	30	487	27	356	20
Alamo-HiSWP[¶]	272	15	272	15	272	15
Alamo-HighGermS[#]	566	32	495	28	341	19

[†] Temperature-Low Dormancy (Details in Table 1.1)
[‡] Alamo-High Germination Parents (Details in Table 1.1)
[§] Kanlow-High Germination Seeds (Details in Table 1.1)
[¶] Alamo-High Seed Weight Parents (Details in Table 1.1)
[#] Alamo-High Germination Seeds (Details in Table 1.1)

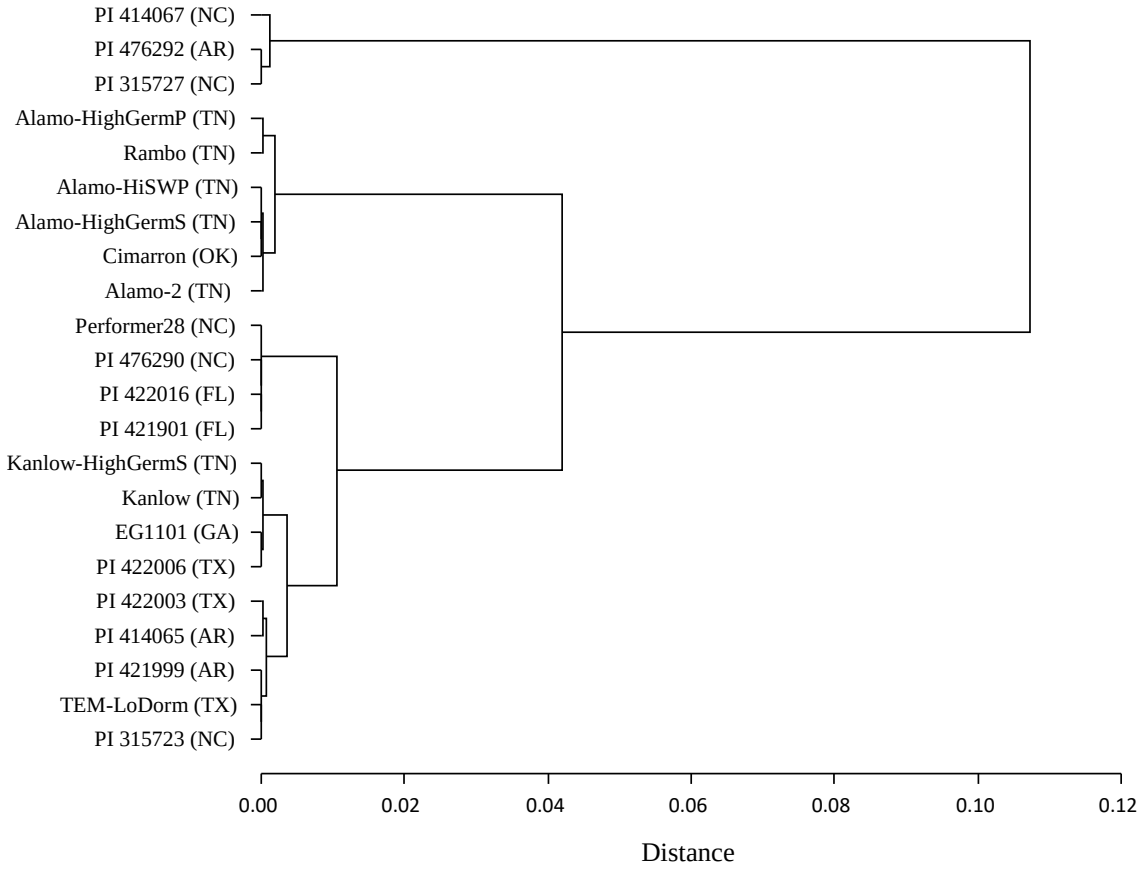

Figure 1.1. Cluster analysis of 22 switchgrass accessions using 8 agronomic traits (biomass yield, days to heading, plant height, tillering ability, stem thickness, spring regrowth, growth habit, and leaf angle) through Ward's minimum variance. Abbreviations: TEM-LoDorm Temperature-Low Dormancy, Alamo-HighGermP Alamo-High Germination Parents, Kanlow-HighGermS Kanlow-High Germination Seeds, Alamo-HiSWP Alamo-High Seed Weight Parents, Alamo-HighGermS Alamo-High Germination Seeds.

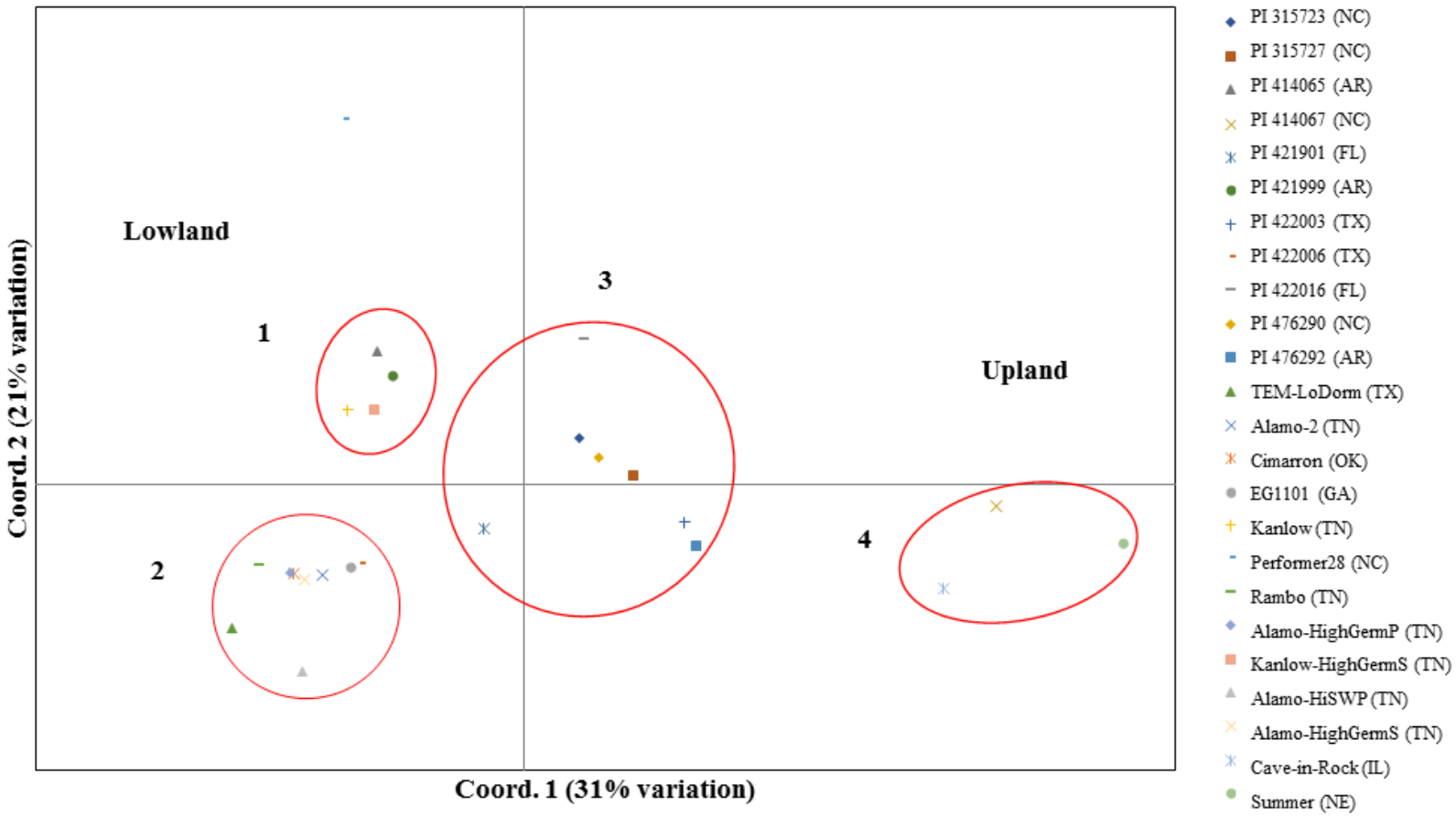

Figure 1.2. Principal Coordinate Analysis (PCoA) of the 24 switchgrass accessions based on the genotyping result from 60 SSR markers where Kanlow and Kanlow derived populations formed group 1, Alamo and Alamo derived accessions formed group 2, an accession from Arkansas, two accessions from Florida, 3 accessions from North Carolina, and one from Texas formed group 3, and upland ecotypes formed group 4. Abbreviations: TEM-LoDorm Temperature-Low Dormancy, Alamo-HighGermP Alamo-High Germination Parents, Kanlow-HighGermS Kanlow-High Germination Seeds, Alamo-HiSWP Alamo-High Seed Weight Parents, Alamo-HighGermS Alamo-High Germination Seeds.

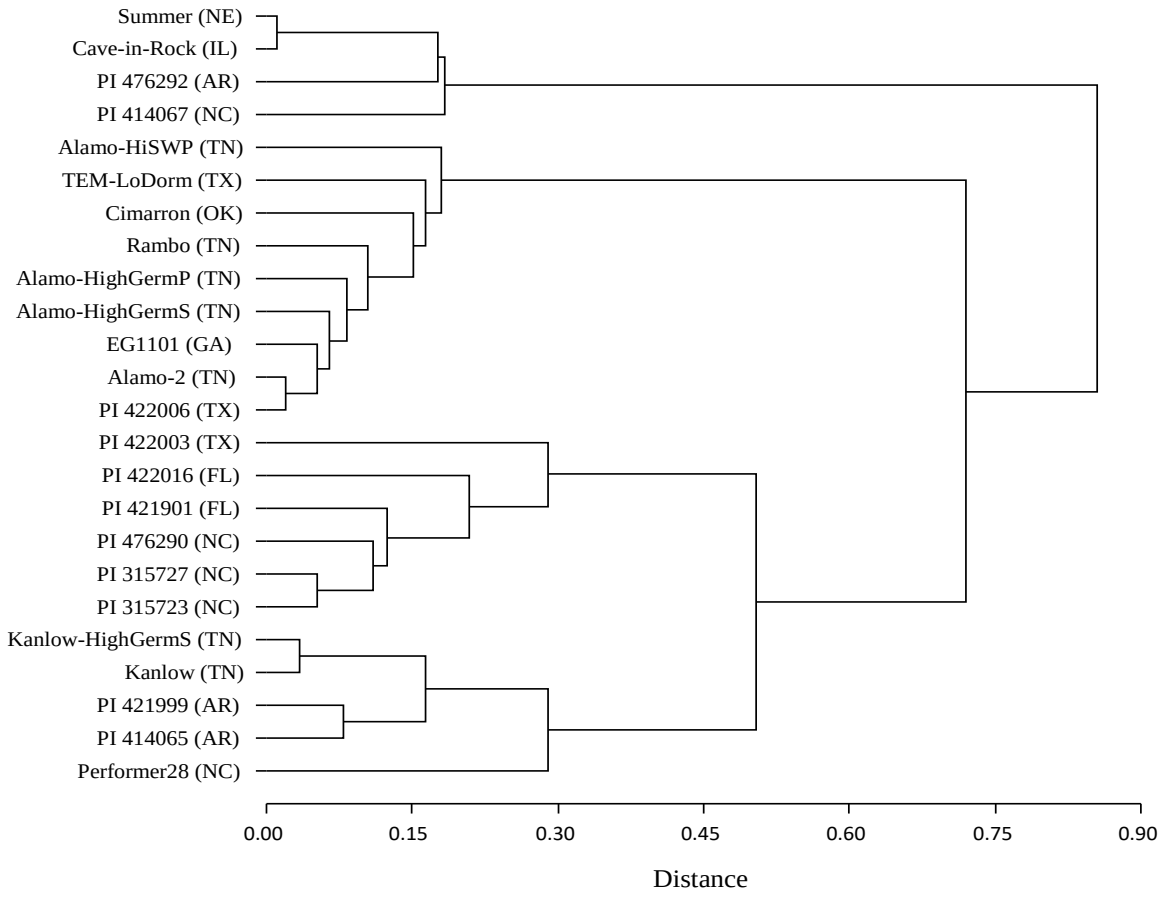

Figure 1.3. Cluster analysis dendrogram of 24 switchgrass accessions using presence or absence of alleles from 60 SSR markers through Ward's minimum variance. Abbreviations: TEM-LoDorm Temperature-Low Dormancy, Alamo-HighGermP Alamo-High Germination Parents, Kanlow-HighGermS Kanlow-High Germination Seeds, Alamo-HiSWP Alamo-High Seed Weight Parents, Alamo-HighGermS Alamo-High Germination Seeds.

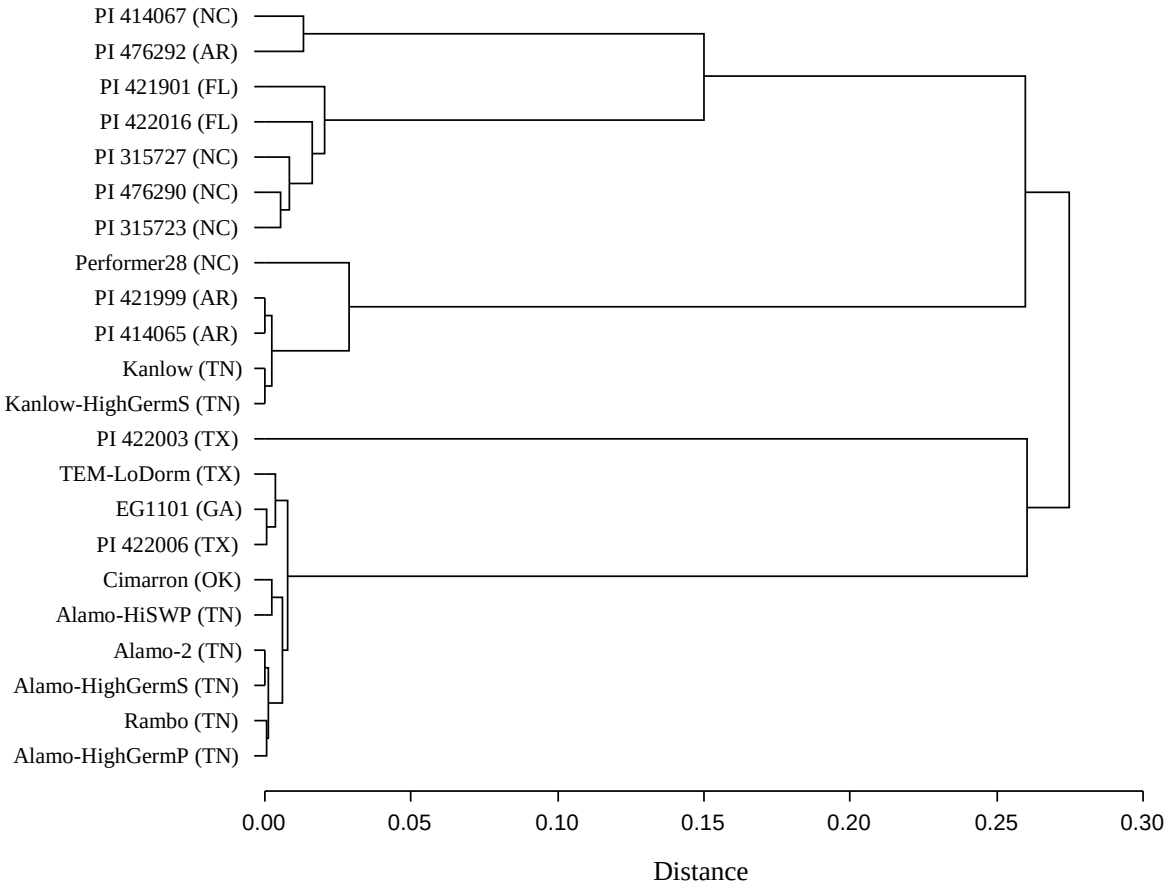

Figure 1.4. Cluster analysis using of 22 switchgrass accessions using the combined genotypic data from 60 SSR markers and phenotypic data from 8 agronomic traits through Ward's minimum variance. Abbreviations: TEM-LoDorm Temperature-Low Dormancy, Alamo-HighGermP Alamo-High Germination Parents, Kanlow-HighGermS Kanlow-High Germination Seeds, Alamo-HiSWP Alamo-High Seed Weight Parents, Alamo-HighGermS Alamo-High Germination Seeds.

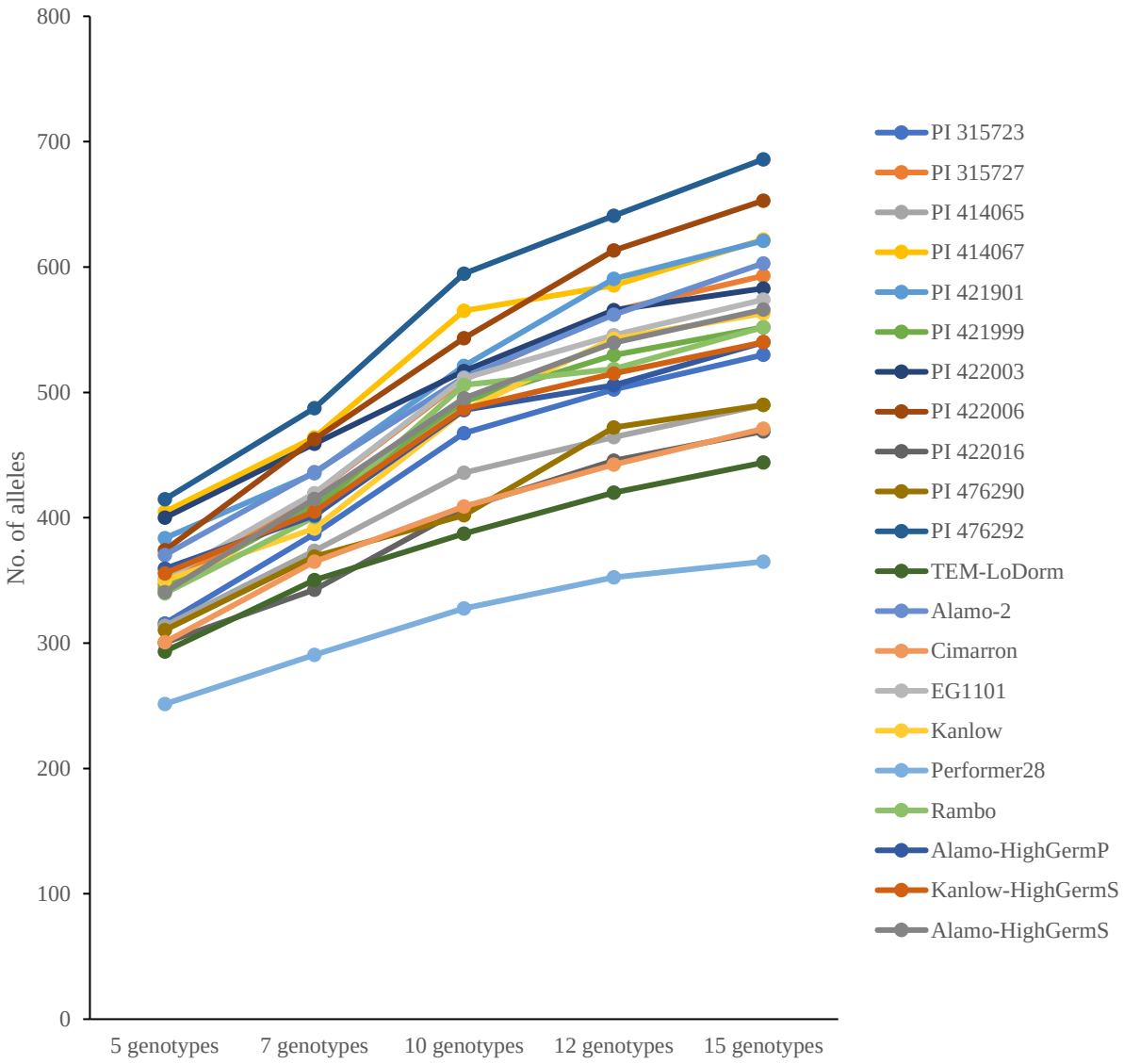

Figure 1.5. Change in the number of alleles detected using different sample sizes. Abbreviations: TEM-LoDorm Temperature-Low Dormancy, Alamo-HighGermP Alamo-High Germination Parents, Kanlow-HighGermS Kanlow-High Germination Seeds, Alamo-HiSWP Alamo-High Seed Weight Parents, Alamo-HighGermS Alamo-High Germination Seeds.

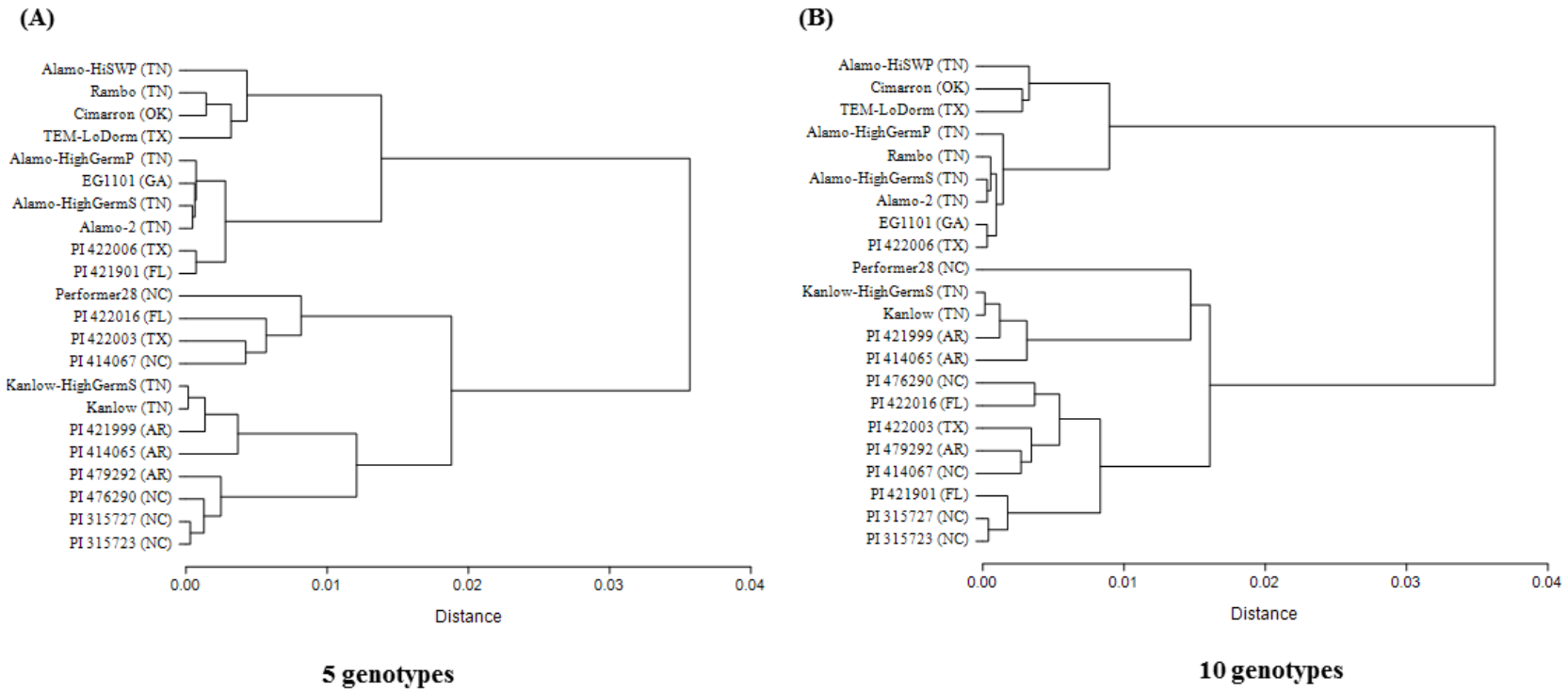

Figure 1.6. Cluster analysis of genotypic data of the 22 lowland switchgrass accessions using different sample sizes through Ward's minimum variance. Abbreviations: TEM-LoDorm Temperature-Low Dormancy, Alamo-HighGermP Alamo-High Germination Parents, Kanlow-HighGermS Kanlow-High Germination Seeds, Alamo-HiSWP Alamo-High Seed Weight Parents, Alamo-HighGermS Alamo-High Germination Seeds.

CHAPTER 2

GENETIC VARIATION AND EXPECTED BIOMASS YIELD GAIN PER CYCLE OF SELECTION IN LOWLAND SWITCHGRASS

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Cheryl O. Dalid conducted the experiments, collected and analyzed the data, and wrote the manuscript.

ABSTRACT

Switchgrass (*Panicum virgatum* L.), a native of the North American prairies, has been selected for bioenergy research. With a focus on biomass yield improvement, this study aims (i) to estimate the genetic variation in biomass yield and important agronomic traits in 'Alamo', (ii) to determine correlations between biomass yield and agronomic traits, and (iii) to compare efficiency of phenotypic selection from a sward plot and advanced cycle half-sibs (ACHS) on the basis of space-plant performance. Sixty-two Alamo half-sib families (AHS) from a 4-yr-old Alamo sward and 20 advanced cycle half-sib families (ACHS) were evaluated in replicated field trials under simulated swards in Knoxville and Crossville, TN. Results showed significant variation ($P < 0.05$) among AHS for biomass yield, tillering ability, and spring vigor, suggesting the importance of additive genetic variation in these traits. Overall mean biomass yield of AHS was not different from the Alamo control, demonstrating the inefficiency of phenotypic selection from swards. Mean biomass yield of ACHS was 15 and 20% less than that of the control and AHS, respectively. Such results could be attributable to the influence of environment and genotype × environment interaction. However, results showed great potential for biomass yield improvement through selection on the basis of family performance. Using 10% selection intensity, parental control of two, and a narrow-sense heritability estimate of 0.11, gain per cycle selection from half-sib family

selection is estimated to be 23%. Spring vigor showed potential use for indirect selection due to its high genetic correlation (r_G = 0.75) with biomass yield. However, it is impeded by the low heritability estimate (h^2 = 0.34).

Abbreviations: ACHS, advanced cycle half-sib family; AHS, ‘Alamo’ half-sib family; AWF, among-and-within-family; ETREC, East Tennessee Research and Education Center; HS, half-sib; r_G, genetic correlation; r_P, phenotypic correlation.

INTRODUCTION

The rapidly depleting supply of fossil fuels and the concern over global climate change due to emissions from their use have led to the quest for alternative clean renewable sources of energy (McLaughlin, 1992). Biofuels from plant biomass feedstock are promising sources of renewable energy that could offer economic opportunities to farmers and have positive effects on the environment (McLaughlin *et al*., 1999). However, potential competition between bioenergy feedstock crops and food crops for arable lands may cause food security problems as a consequence (Tenenbaum, 2008; Gamborg *et al*., 2012). Thus, ideal bioenergy feedstock species are ones that can be successfully grown under marginal environments. Switchgrass (*Panicum virgatum* L.), a native perennial grass of the United States, has high biomass yield potential, can grow in marginal lands, and has the ability to sequester a large amount of atmospheric CO_2 into the soil (Blanco-Canqui, 2010). Due to these advantages, switchgrass was chosen as the candidate herbaceous species for bioenergy feedstock production in the United States (McLaughlin, 1992; McLaughlin *et al*., 1999; Sanderson *et al*., 2006; Wright and Turhollow, 2010).

Switchgrass is a warm-season C_4 grass adapted to a wide range of ecosystems in North America, ranging from Mexico to Canada (Stubbendieck *et al*., 1997). Native populations of switchgrass are classified into two ecotypes, lowland and upland (Hultquist *et al*., 1996). These ecotypes are also associated with the different cytoplasm types, "L" for lowland and "U" for upland, which was determined through chloroplast DNA polymorphisms (Hultquist *et al*., 1996; Vogel, 2004; Missaoui *et al*., 2006). The two ecotypes differ greatly in terms of their morphology and adaptation (Vogel and Burson, 2004). Lowland ecotypes are adapted to the southern wetlands, grow vigorously, flower late in the season, are tall and thick stemmed with long and wide leaves, and produce high biomass yield. Upland ecotypes are adapted to the northern dryland, are thin

stemmed, have short plants and fine leaves, and produce less biomass (Moser and Vogel, 1995; Vogel, 2004). The two ecotypes also differ in ploidy levels: lowland ecotypes are almost exclusively tetraploids ($2n = 4x = 36$), whereas upland ecotypes are mostly octaploids ($2n = 8x = 72$) and a few are tetraploids (Gunter *et al*., 1996; Casler *et al*., 2011; Zhang *et al*., 2011). Segregation of a large proportion of molecular markers supports disomic inheritance in lowland switchgrass (Okada *et al*., 2010).

Switchgrass is an allogamous species, which is primarily due to its S-Z gametophytic self-incompatibility system (Martínez-Reyna and Vogel, 2002). As a result, populations of switchgrass, both native and cultivated, are highly heterogeneous, and individual plants are highly heterozygous (Parrish and Fike, 2005). Genetic improvement and breeding of new cultivars are the most effective and inexpensive way of increasing biomass yield (Bartley *et al*., 2013). Common switchgrass breeding methods include recurrent phenotypic selection with some restrictions and among-and-within-family (AWF) selection methods (Vogel and Burson, 2004**;** Casler and Brummer, 2008). The potential of exploiting biomass heterosis in crosses between ecotypes or between strains within ecotypes have been documented (Vogel and Mitchell, 2008; Casler, 2014; Bhandari *et al*., 2017).

Genetic components of variation and heritability estimates are important parameters for quantitative trait improvement. Understanding trait heritability is important in determining the ideal breeding strategy. In a study by Talbert *et al*. (1983), the narrow-sense heritability estimates for biomass yield in lowland switchgrass based on half-sib (HS) family variation were 0.25 using individual plant data and 0.59 using plot means. Bhandari *et al*. (2011) estimated narrow-sense heritability for biomass yield of lowland switchgrass in a space-planted population as 0.17 based on variation among HS families and 0.24 based on parent-progeny regression. Such variation in

heritability estimates for biomass yield likely reflects the use of different reference populations and environmental conditions, and the estimation methods. In general, these estimates using single-plant data are considered low, suggesting the complex genetics and environmental variance involved in biomass yields.

Improvement of quantitative traits with low heritability can be very challenging, and the selection progress can be slow. Breeders sometimes look for secondary traits that can be used as an indirect method for selection of primary traits (Bhandari *et al*., 2011; Casler, 2012). In a space-plant evaluation of full-sib families by Bhandari *et al*. (2011), biomass yield was found to be significantly correlated with plant height (phenotypic correlation [r_P] = 0.56), stem thickness (r_P = 0.52), tillering ability (r_P = 0.45), and days to flowering (r_P = 0.46). They also reported a positive r_P between plant height and stem thickness (r_P = 0.77). These relationships were consistent with the results reported by Das *et al*. (2004). Further research is needed to investigate if some of these secondary traits also have high genetic correlations with biomass yield to enable use in indirect selection.

Most of the genetic studies performed in the past were conducted in space-planted nurseries where plant spacing ranged from 1 to 2.25 m^2 (Bhandari *et al*., 2013; Sykes *et al*., 2017). In contrast, switchgrass growers use drills to establish the crop. Recent studies indicated that plant performance under space-plant nursery has low predictive value for biomass yield under sward condition (Sykes *et al*., 2017). Ideally, selection nurseries should be established using drill plots. However, differences in seed dormancy and difficulty in preventing weeds are challenges that hinder uniform plant density in drill-plot experiments. Appropriate plant spacing in breeding nurseries also facilitates individual plant selection. Here, we present the genetic parameters estimated based on variation among HS families that were evaluated under moderately high plant

density. ‘Alamo’ was used as a base population because it is the most widely adapted population in the south and southeastern regions and has high biomass yield potential.

The objectives of the current study were (i) to assess genetic variation in biomass yield and other important agronomic traits of the Alamo population and estimate narrow-sense heritability and expected gain from selection, (ii) to determine correlations between biomass yield and agronomic traits, and (iii) to evaluate the efficiency of phenotypic selection from a sward plot on the basis of plant vigor and among-and-within-HS-family selection on the basis of space-plant evaluation. Here, we present estimation of these parameters using HS progeny performance under high plant density.

MATERIALS AND METHODS

Plant Materials

Selection on the Alamo population of switchgrass was initiated in 2011 using a 4-yr-old sward that was previously established for other agronomic studies. The seed used to establish the Alamo sward was purchased from ERNST Seeds (Meadville, PA). The sward, 1.2 ha in size, was established in 2007 at the Holston Unit of the East Tennessee Research and Education Center (ETREC). In fall 2011, 230 individual plants were selected on the basis of plant vigor (i.e., visual observation of plant height, tiller number, and tiller mass at maturity). Open-pollinated seeds were collected from 10 panicles per selected genotype to generate Alamo HS families (AHS). After threshing and cleaning, 96 AHS families with >300 seeds were retained for further evaluation. The original seed lot of Alamo, hereafter referred to as "Alamo C0," was included as a check.

Twenty advanced cycle HS families (ACHS) were also included for comparison. These ACHS families were derived through AWF selection from an ongoing breeding nursery at the time. The nursery consisted of 72 HS families of lowland switchgrass that were previously generated in 2008 by polycrossing the genotypes of five lowland populations of switchgrass including three improved populations ('Cimarron', NSL2001-1, and PI 607837) and two plant introduction accessions (PI 421999 and PI 422016). Cimarron, a cultivar derived from Alamo and 'PMT 279', was developed and released by Oklahoma State University. Improved population NSL2001-1 was developed at Oklahoma State University through selection on a broad-base population comprising several southern and northern lowland collections. The PI 607837 was developed at the Grassland, Soil, and Water Research Laboratory in Temple, TX, after two cycles of recurrent restricted phenotypic selection on an Alamo population. Accessions PI 421999 and PI

422016 were landraces collected from Arkansas and Florida, respectively. Each of these population sources was represented by 14 genotypes in the polycross. The polycross was established in 2007, and open-pollinated HS seeds were collected in 2008. The HS family plot was established at the ETREC Holston Unit in 2009. It was a space-plant nursery (1.2 × 1.2 m) where 10 plants per HS were evaluated. Year 2 biomass yield was recorded in 2010. Using among-family selection, the highest yielding 20 plants were selected from 14 superior-performing HS families. At the end of fall 2011, open-pollinated seeds were harvested from the selected genotypes, generating 20 ACHS families included in the current study for comparison.

Seed Germination and Seedling Preparation

Seed dormancy treatment, germination, and seedling preparation followed the procedure described by Bhandari *et al*. (2011). Seed of the 96 newly derived AHS, 20 ACHS, and the Alamo C0 check were scarified by immersing in 100% household bleach (5.25% sodium hypochlorite solution) for 15 min, followed by rinsing twice with tap water. Seeds were germinated in Petri dishes at room temperature. After 2 wk, germinated seedlings were potted in 72-well flats filled with greenhouse soil, Metromix 300, and the seedlings were grown in the greenhouse for 13 weeks. Given the number of seedlings available at the time of planting, 62 AHS, all the 20 ACHS, and Alamo C0 were retained for field evaluation.

Field Design and Data Collection

The field nursery was established in spring 2012 at two Tennessee locations—the ETREC Holston Unit in Knoxville, TN (35°58′42″ N, 83°51′28″ W), and the Plateau Research and Education Center in Crossville, TN (36°00′56.3″ N, 85°07′56.0″ W). Soil types were a Shady-Whitwell complex (fine-loamy, mixed, subactive, thermic Typic Hapludults; fine-loamy,

siliceous, semiactive, thermic Aquic Hapludults) at the Knoxville site and Lonewood loam (fine-loamy, siliceous, semiactive, mesic Typic Hapludults) at the Crossville site. The seedlings were transplanted on 31 May and 7 June at Knoxville and Crossville sites, respectively. The experiment was established using a randomized complete block design with three replications. Each family in each replication was planted in a single-row plot of nine plants. Rows were spaced 0.9 m, and within-row plant spacing was 0.3 m. This was an experiment with moderately high plant density (0.27 m^2 $plant^{-1}$) compared to the plant spacing (1–2.26 m^2 $plant^{-1}$) conventionally used in most selection nurseries of perennial grasses (Humphreys, 1989; Vogel and Pedersen, 2010). This is to ensure sufficient plant competition and facilitate individual plant selection. Fertilizer was not applied during the establishment year. In each spring of the post establishment years, 60 kg N ha^{-1} was applied. Preemergence herbicides Prowl H_2O (Pendimethalin, BASF Corporation) at 3.31 L ha^{-1} and Dual II Magnum (Metolachlor, Syngenta Crop Protection) at 2.84 L ha^{-1} were applied in spring of each year. About 60 d after transplanting, postemergence herbicide Accent (Du Pont) at 18.9 g ha^{-1} with crop oil (1% v/v spray solution) was applied at the Knoxville site to control nutsedges (*Cyperus* spp.). At the Crossville site, 2,4-D (2,4-dichlorophenoxyacetic acid) at 2.37 L ha^{-1} with surfactant at 1.18 L ha^{-1} was applied postemergence to control ragweed (*Ambrosia* spp.). Minimal irrigation was applied during the first 60 d after transplanting. The plot was mowed at the end of the fall after the first killing frost. Agronomic traits such as plant height, stem thickness, and tiller count were recorded from individual plants during fall 2013 and 2014. Plant height was measured from the base of the plant to the tip of the panicle prior to harvest. Stem thickness from individual plants was scored from 1 to 5, with 1 being the thinnest stem and 5 being the thickest. Tillers were counted from two replications in both locations during 2013. In 2014, tillering ability was scored using a scale of 1 to 9 due to time constraints. For combined analysis, 2013 tiller count

data were converted to scores 1 to 9, with 1 being a tiller count <10 and 9 being a tiller count >90. Spring vigor was scored from 1 to 5, with 1 being the least vigorous and 5 being the most vigorous. Spring regrowth was recorded according to the Julian calendar.

Biomass yield was recorded in fall 2013 (Year 2 growth) and 2014 (Year 3 growth) several weeks after the killing frost. In Crossville, biomass was harvested both years on 15 December. In Knoxville, 2013 biomass was harvested on 19 November and 2014 biomass was harvested on 22 Jan. 2015. Biomass was harvested using a Hege forage chopper (Wintersteiger) in Crossville, whereas in Knoxville, a Carter Flail-type harvester (Carter Manufacturing Company) was used. About 200 g of biomass per plot was sampled from two replications for moisture content. Samples were dried in a batch oven (Wisconsin Oven Corporation) for 72 h at 49°C. Sample fresh weight and dry weight were obtained, and moisture content at harvest was determined and was used to obtain the dry matter yield of the corresponding entries in each location.

Data Analysis

Genetic Variation and Heritability

Data on biomass yield and yield components were analyzed using the MIXED model in SAS (SAS Institute, 2013). Location and year were fixed effects, and replication and HS family were used as random factors in the model. Genetic variation among AHS was estimated using plot mean data recorded for 2013 and 2014 in both locations. Variation among plants within families was estimated from 2013 biomass yields of individual plants estimated for five plants per plot. Five tillers were sampled, and dry matter yields were recorded and multiplied to total tillers to obtain yields of corresponding plants. Least square means for locations and years were obtained, and their statistical differences determined according to Fisher's protected LSD ($P < 0.05$).

Narrow-sense heritability for biomass yields and yield components were estimated according to the component of variation among HS families (Gallais, 2003) using the equation by Eberhart and Newell (1959):

$$h^2 = \sigma_A^2/\sigma_P^2 = \frac{4\,\sigma_{hs}^2}{\sigma_{hs}^2 + \sigma_{hs\,x\,L}^2 + \sigma_{hs\,x\,Y}^2 + \sigma_{hs\,x\,L\,x\,Y}^2 + \sigma_{w(hs)}^2}$$

where σ_A^2 is the additive variance; σ_P^2 estimated total phenotypic variance; σ_{hs}^2 , $\sigma_{hs\,x\,L}^2$, $\sigma_{hs\,x\,Y}^2$, and $\sigma_{hs\,x\,L\,x\,Y}^2$ are the variance estimates for HS families and its interaction with location, year, and location × year, respectively, and $\sigma_{w(hs)}^2$, is the variance among plants within half-sib families. The SEs for heritability were estimated according to Hallauer *et al*. (2010).

Variance components were estimated using the restricted maximum likelihood of the MIXED procedure (SAS Institute, 2013). Expected genetic gain was estimated for the selection of 10 and 15% superior HS families and parental control of one and two. The expected genetic gains were estimated using following equation:

$$\Delta G = ckh^2\sigma_P$$

where c is the parental control, k is the selection intensity (Hallauer *et al*., 2010), h^2 is narrow sense heritability, and σ_P is the phenotypic standard deviation (Nguyen and Sleper, 1983). Parental control value is 1 when remnant seed of selected HS families is used as recombination units, and this value is 2 when parental clones are used as recombination units.

Phenotypic and Genetic Correlations

The phenotypic and additive genetic correlations were generated using restricted maximum likelihood -based estimation in the MIXED procedure in SAS, as described by Holland (2006). The genetic correlations were estimated as follows:

$$r_{G(trait1,trait2)} = \sigma_{G(trait1,trait2)} / \sqrt{\sigma^2_{G(trait1)}\sigma^2_{G(trait2)}}$$

where $r_{G(trait1,trait2)}$ is the genetic correlation between two desired traits and $\sigma_{G(trait1,trait2)}$ is the genetic covariance of trait 1 and trait 2, $\sigma^2_{G(trait1)}$ is the genetic variance of trait 1, and $\sigma^2_{G(trait2)}$ is the genetic variance of trait 2.

Mean Comparisons

Mean biomass yields of AHS, ACHS, and Alamo C0 from each year, each location, and across years and locations were generated using the MIXED model (SAS Institute, 2013). Single-df contrasts were used to compare the group means of AHS, ACHS, and Alamo C0. If the phenotypic selection from a sward was effective, it was expected that AHS would have higher mean biomass yield than Alamo C0. Similarly, if AWF selection in a spaced-plant evaluation was effective, ACHS was expected to produce a higher mean biomass than AHS.

RESULTS AND DISCUSSION

Genetic Variation and Narrow-Sense Heritability

Alamo half-sibs differed in their mean biomass yield ($P<0.05$) across two locations (Knoxville and Crossville) and years (2013 and 2014), which suggested an important role of additive genetic variation in biomass production (Table 2A.1). Biomass yield was also influenced by AHS × year interaction ($P<0.05$). However, AHS interactions with location and location × year were not evident. There was significant variation among genotypes within AHS families ($P<0.01$, Table 2A.1, Figure 2A.1). The fixed effects of location and year were significant ($P<0.05$), with the year effect having a higher value. Analysis by location showed that biomass yield varied among ($P<0.05$) and within AHS ($P<0.01$) in both locations.

In terms of the agronomic traits observed, AHS families differed in their tillering ability ($P< 0.05$) and spring vigor (Table 2A.2). Results did not show significant variation among AHS in plant height, stem thickness, and days to spring regrowth. There was no influence of AHS interaction with location, year, and location × year for any of these traits. However, genotypes within AHS varied widely for all the agronomic traits recorded. All these traits were found to be influenced by location, year, and location × year interaction.

The means and ranges for biomass yield and other agronomic traits are presented in Table 2A.3. Mean biomass yields ranged from 11.07 to 19.22 t ha^{-1}. Two-year mean biomass yield at the Knoxville site ranged from 9.09 to 18.85 t ha^{-1}. At the Crossville site, the mean biomass yield ranged from 11.11 to 20.49 t ha^{-1}. Mean tillering ability score ranged from 3 to 5. Mean spring vigor score, on the other hand, which was only recorded at Knoxville in 2013, ranged from 2 to 5.

These values confirm the significant variation in AHS biomass yield, tillering ability, and spring vigor that were detected in the ANOVA. Means for plant height, stem thickness, and spring regrowth recorded for AHS were 236 cm, 3, and 88 d (Julian calendar), respectively.

Significant genetic variation in the Alamo population of switchgrass for biomass yield, tillering number, and spring vigor demonstrated the potential for improvement of these traits. The poor heritability of biomass yield warrants rigorous family-performance-based selection. The component of variation within AHS was of much higher magnitude than that among AHS (Table 2A.1). This holds true for the five other agronomic traits measured, although plant height, stem thickness, and spring regrowth were not different among AHS. The high within-family variation was not surprising given the fact that among-AHS variation accounts for one-fourth of the additive genetic variation, whereas within-AHS variation includes three-fourths of the additive as well as the dominant genetic variation. Casler and Brummer (2008) have demonstrated potentials for improvement in expected genetic gain using AWF selection. However, researchers need to be aware of the potential confounding effects of the nonadditive component of genetic variation and the residual variation. The influence of residual variation on selection can be reduced by planting clonal replicates that will parse out some of the residual variation associated with single-plant data and improve AWF selection efficiency.

Effects of environments and genotype × environment interactions on plant performance are common in most crop species (Bernardo, 2014; Brown *et al*., 2014). In the current study, significant AHS × year interaction was evident only at Knoxville site (Table 2A.1). This could be due to spring frost that occurred in 2014 that could have selectively pushed back early breaking families. The effect of the year reflects the fact that perennial grasses during their initial years of

growth invest a significant amount of resources in root development. In switchgrass, full biomass yield potential can be realized during the third production year (McLaughlin *et al*., 1999).

Estimation of Narrow-Sense Heritability and Expected Selection Gain

Narrow-sense heritability estimates were 0.11 for biomass yield (Table 2A.1), 0.13 for tiller number, and 0.34 for spring vigor (Table 2A.2). The low narrow-sense heritability for biomass yield computed from variance components was comparable with heritability estimates previously reported for lowland switchgrass (Talbert *et al*., 1983; Rose *et al*., 2008; Bhandari *et al*., 2011). These low heritability estimates for biomass yield and its component traits indicated the likely challenges of improving these traits. Heritability estimates for plant height, stem thickness, and spring regrowth were not possible because of nonsignificant variation among AHS for these traits.

The expected gain from selection was computed using the heritability estimate for biomass yield with the selection of 10 and 15% superior families (Table 2A.4). Selection based on HS progeny performance resulted in a significant expected improvement in biomass yield. With a 15% selection pressure and a parental control of one, such as when using remnant seeds of selected HS families as recombination unit, a gain of 1.53 t ha^{-1} (10.4%) is expected. The expected gain is doubled (i.e., 3.05 t ha^{-1}, a 20.6% gain) when parental clones of half-sibs are used as recombination unit. Selection gain can be further improved to 23.4% when only 10% of the superior families are selected (Table 2A.4). Such gain is comparable with the biomass yield gain observed in switchgrass and bahiagrass (*Paspalum notatum* Flueggé) (Burton, 1982; Bhandari *et al*., 2013).

It is a common practice to use <10 clones to produce synthetic varieties of forage grasses (Wu and Taliaferro, 2009; Bhandari *et al*., 2013). If a breeder plans to use the same population in

the repeated cycle of recurrent selection, reduced genetic variation could be a limiting factor. Increasing the number of HS families evaluated in each cycle and adopting AWF selection would ensure greater genetic variation in breeding populations.

Phenotypic and Genetic Correlations

Phenotypic and genetic correlations between biomass yield and important agronomic traits were determined using the 62 AHS families. Results showed a significant but low positive r_P of biomass yield with tillering ability ($r_P = 0.33 \pm 0.05$) and plant height ($r_P = 0.35 \pm 0.05$), and a moderately strong positive correlation was observed between biomass yield and spring vigor ($r_P = 0.75 \pm 0.04$). The positive correlations of biomass yield with tiller number and plant height were also reported by Das *et al*. (2004) and Bhandari *et al*. (2011). To further examine the importance of trait relationships, genetic correlations were also determined. Biomass yield showed strong positive genetic correlations with tillering ability ($r_G = 0.67 \pm 0.14$), plant height ($r_G = 0.70 \pm 0.15$), and spring vigor ($r_G = 0.76 \pm 0.20$).

Correlations between traits can be useful in crop improvement, as it opens the possibility of indirect selection, especially when the main trait of interest has complex genetics and has poor heritability (Hallauer *et al*., 2010). Indirect selection can be advantageous when the heritability of the indirect trait is higher than that of the primary trait and indirect trait has a strong genetic correlation with the primary trait (Hallauer *et al*., 2010; Casler, 2012). Although the positive genotypic correlation of biomass yield with tillering ability was promising, relatively low heritability estimates for tillering ability ($h^2 = 0.13$) indicate that tillering may not necessarily be a good candidate for indirect selection. This is in contrast with previous results showing significant contribution of tillering ability to biomass yield (Das *et al*., 2004; Bhandari *et al*., 2011). The

variation in estimates may reflect the fact that earlier results were based on experiments that were conducted using wider plant spacing (1-2.25 m^2). Current results are estimated according to the experiment that was conducted in high plant density (0.27 m^2) that may better simulate the production conditions. Spring vigor has a moderately strong positive genetic correlation (r_G = 0.76 ± 0.20). However, its potential for use in indirect selection may be constrained due to the low heritability (h^2 = 0.34).

Efficiency of Phenotypic Selection from Sward

The AHS evaluated in this study represented the open-pollinated seeds harvested from plants that were selected from a 4-yr-old sward, and the selection was based on phenotypic vigor at maturity. If the phenotypic selection was effective, AHS would have a higher mean biomass yield than the Alamo C0 check. However, results did not show any gain from phenotypic selection. Indeed, biomass yield of AHS (14.77 t ha^{-1}) was numerically lower than biomass yield recorded for Alamo C0 (15.72 t ha^{-1}) (Table 2A.5), and the results were consistent across locations and years. This demonstrated the inefficiency of phenotypic selection from swards, which likely suggests the complex genetics underlying biomass yield and sensitivity of individual switchgrass genotypes to soil heterogeneity. There were 4% of the AHS individual plants that were higher than the highest yielding Alamo plant, but these individual plant data were only estimates from a sample of five plants, and the individual plants from the two locations were not clonal replicates.

Yield Comparison: Alamo Half-Sib Families vs. Advanced Cycle Half-Sib Families

Twenty ACHS included in the current study represented open-pollinated seeds collected from 20 plants that were selected from 72 lowland HS families using an AWF selection procedure (Brummer and Casler, 2009). As described above, the selection was based on Year 2 biomass yield

performance under space-plant (1.25 × 1.25 m) evaluation, and 20 most vigorous plants from 14 superior half-sibs were selected. The mean biomass yield for ACHS (12.59 t ha^{-1}) was 15 and 20% less than the mean biomass yield recorded for AHS (14.77 t ha^{-1}) and Alamo C0 (15.72 t ha^{-1}), respectively ($P < 0.05$) (Table 2A.5), and the results were consistent across test locations and years. The results could be attributable to the high environment influence on single-plant performance and high genotype × environment interaction in switchgrass (Hopkins *et al*., 1995; Casler and Boe, 2003).

Earlier results have questioned the efficiency of phenotypic selection in switchgrass (Vogel and Mitchell, 2008; Price and Casler, 2014; Sykes *et al*., 2017). If the phenotypic selection was effective, AHS would have higher mean biomass yield than Alamo C0. However, no difference in biomass production between AHS and Alamo C0 further confirms earlier findings. It was counterintuitive to observe that ACHS would perform poorly as compared with both Alamo C0 and AHS despite the fact that three of the five populations that were used to generate ACHS base population were released or experimental cultivars, and ACHS was derived following the AWF procedure. Such a lack of yield gain in ACHS could reflect the inefficiencies of selection under space-plant conditions, as well as the larger influence of genotype × environment interactions on single-plant performance. Sykes *et al*. (2017) reported a negative correlation between plant performance under spaced-plant and sward conditions. Bhandari *et al*. (2013) reported a higher selection gain from selection among HS families based on performance under sward compared with AWF selection based on performance under space-plant conditions. Strong genotype × environment interaction may have also contributed to reduced yield of ACHS family despite the fact that three of the five populations that were used to generate the ACHS base population were

improved cultivars with higher biomass yield in their corresponding selection environments (Bhandari *et al*., 2013).

In summary, the Alamo population shows a significant variation in biomass yield and offers potential for genetic improvement. The poor heritability of biomass yield warrants rigorous family-performance-based selection. A large genetic variation among genotypes within families demonstrated the potential for improving genetic gain using AWF selection. However, due to extreme influence of environment on single-plant performance, it may be advisable to consider evaluating at least two clonal replicates of each genotype within HS; thus, the environmental influence on AWF selection can be separated and the genetic selection gain can be improved. As suggested by Casler *et al*. (2007), adopting the regional breeding program based on hardiness zone and well-characterized ecoregions would help address issues of genotype × environment interactions.

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APPENDIX 2A

CHAPTER 2 TABLES AND FIGURES

Table 2A.1. Components of variation of the 62 Alamo half-sib families (AHS) for biomass yield (t ha^{-1}) across locations during 2013 and 2014.

		Estimates of variance components		
Source of variation	df	Knoxville	Crossville	Combined
Replication (rep)/rep(Location)	2 (4)†	0.00	0.59	0.25
AHS	61	2.36*	2.83*	1.71*
AHS × location	61	–	–	0.86
AHS × rep/rep(location)	122 (244)	4.16***	5.66***	4.95***
AHS × year	61	0.83*	0.53	0.68*
AHS × location × year	61	–	–	0.01
AHS × year × rep(location)	124 (248)	3.71***	6.63***	5.17***
Genotype(AHS)‡	248	77.95***	67.83***	74.53***
		Test of fixed effects (*F* values)		
Location	1	–	–	16*
Year	1	172***	82***	195***
Location × year	1	–	–	2.76
Narrow-sense heritability (h^2)				0.11 (0.31)§

* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
† The df in parentheses is for combined locations.
‡ Estimated from 2013 single-plant yield data of five plants per plot using five tiller samples at maturity; two replications from Knoxville and one from Crossville.
§ SE is given in parentheses.

Table 2A.2. Components of variation of the 62 ‘Alamo’ half-sib families (AHS) for other agronomic traits across locations during 2013 and 2014.

Source of variation	Tillering ability†	Plant height	Stem thickness‡	Spring vigor§	Spring regrowth¶
	Estimates of variance components				
Replication (rep)/rep(location)	0.24	5.00	0.01	0.07	0.62
AHS	0.08*	27.05	0.01	0.22**	0.04
AHS × location	0.00	0.00	0.00	–	0.05
AHS × rep/rep(location)	0.03	90.88***	0.01	0.33***	0.24**
AHS × year	0.00	0.00	0.00	–	–
AHS × location × year	0.00	21.52	0.00	–	–
AHS × year × rep(location)	0.58***	137***	0.24***	0.25	0.56
Genotype(AHS)†	2.14***	561***	1.48***	2.04***	2.86***
	Test of fixed effects (*F* values)				
Location	0.80	4.39	35*	–	823***
Year	205***	138***	62***	–	–
Location × year	27.32***	234***	190***	–	–
Narrow-sense heritability (h^2)††	0.13 (0.45)	–	–	0.34 (0.73)	–

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† Tillering ability is rated as a score from 1 (<10) to 9 (>80).
‡ Stem thickness is rated as a score from 1 (thinnest) to 5 (thickest).
§ Spring vigor is rated as a score from 1 (worst) to 9 (best).
¶ Based on the Julian calendar.
# Estimated from 2013 single-plant yield data of 5 plants per plot using 5 tiller samples at maturity; 2 reps from Knoxville and one rep from Crossville.
†† SE is given in parentheses.

Table 2A.3. Mean and range of 'Alamo' half-sib family (AHS) biomass yield and other agronomic traits within location, within year, and across years and locations.

Trait	Knoxville 2013	Knoxville 2014	Knoxville Combined	Crossville 2013	Crossville 2014	Crossville Combined	2 locations & 2 yr
			Biomass yield				
			t ha^{-1}				
Mean	11.48	15.31	13.42	15.06	17.33	16.17	14.77
Maximum	16.42	21.48	18.85	20.41	22.59	20.49	19.22
Minimum	6.75	10.37	9.09	8.93	11.69	11.11	11.07
LSD	5.43	5.43	4.77	5.43	5.43	4.77	3.33***
			Tilling ability†				
Mean	4	4	4	5	4	5	4
Maximum	6	6	5	6	5	6	5
Minimum	3	2	3	4	2	3	3
LSD	2	2	2	2	2	2	1*
			Plant height				
			cm				
Mean	260.03	217.75	238.89	229.35	235.12	235.12	235.61
Maximum	313.04	260.35	268.77	261.56	268.10	250.53	255.84
Minimum	225.34	183.55	213.80	205.53	211.85	212.22	218.44
LSD	33.66	33.66	33.02	33.66	33.66	33.02	23.26
			Stem thickness‡				
Mean	2	3	3	4	3	3	3
Maximum	3	4	3	5	4	4	4
Minimum	2	2	2	3	2	3	2
LSD	1	1	1	1	1	1	1
			Spring vigor§				
Mean	4	–	4	–	–	–	4
Maximum	5	–	5	–	–	–	5
Minimum	2	–	2	–	–	–	2
LSD	1***	–	1***	–	–	–	1***
			Spring regrowth				
			d in Julian calendar				
Mean	–	78	78	–	99	99	88
Maximum	–	80	80	–	101	101	90
Minimum	–	77	77	–	98	98	87
LSD	–	2	2	–	2	2	1

* Significant at the 0.05 probability level.
*** Significant at the 0.001 probability level.
† Tillering ability is rated as a score from 1 (<10) to 9 (>80).
‡ Stem thickness is rated as a score from 1 (thinnest) to 5 (thickest).
§ Spring vigor is rated as a score from 1 (worst) to 9 (best).

Table 2A.4. Genetic gain based on narrow-sense heritability estimate of biomass yield in 2013.

Selection intensity	Standard selection differential (k)†	Parental control‡	$h^2_{(Biomass)}$§	Expected gain
%				t ha^{-1}
10	1.76	1	0.11 (0.31)	1.72
		2	0.11 (0.31)	3.45
15	1.55	1	0.11 (0.31)	1.53
		2	0.11 (0.31)	3.05

† Hallauer *et al*. (2010).
‡ Parental control = 1 when using remnant seeds of selected half-sibs; parental control = 2 when using parental clones of selected half-sibs.
§ SE is given in parentheses.

Table 2A.5. Mean biomass yield (and range) of ‘Alamo’ half-sib family (AHS), advanced cycle half-sib families, and the Alamo C0 check.

	Knoxville		Crossville		
Population	2013	2014	2013	2014	Combined
	t ha^{-1}				
AHS	11.48 (6.75–16.42)	15.31 (10.37–21.48)	15.06 (8.93–20.41)	17.33 (11.69–22.59)	14.77 (11.07–19.22)
ACHS	9.18 (4.9–12.06)	12.14 (6.21–16.95)	13.70 (9.26–19.26)	15.43 (6.05–23.91)	12.59 (6.54–16.67)
Alamo	10.08 (–)	17.08 (–)	16.87 (–)	18.85 (–)	15.72 (10.08–18.85)
	Single-df contrast test				
AHS vs. ACHS	***	***	**	**	***
AHS vs. Alamo	ns†	ns	ns	ns	ns
ACHS vs. Alamo	ns	**	ns	ns	*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† ns, not significant.

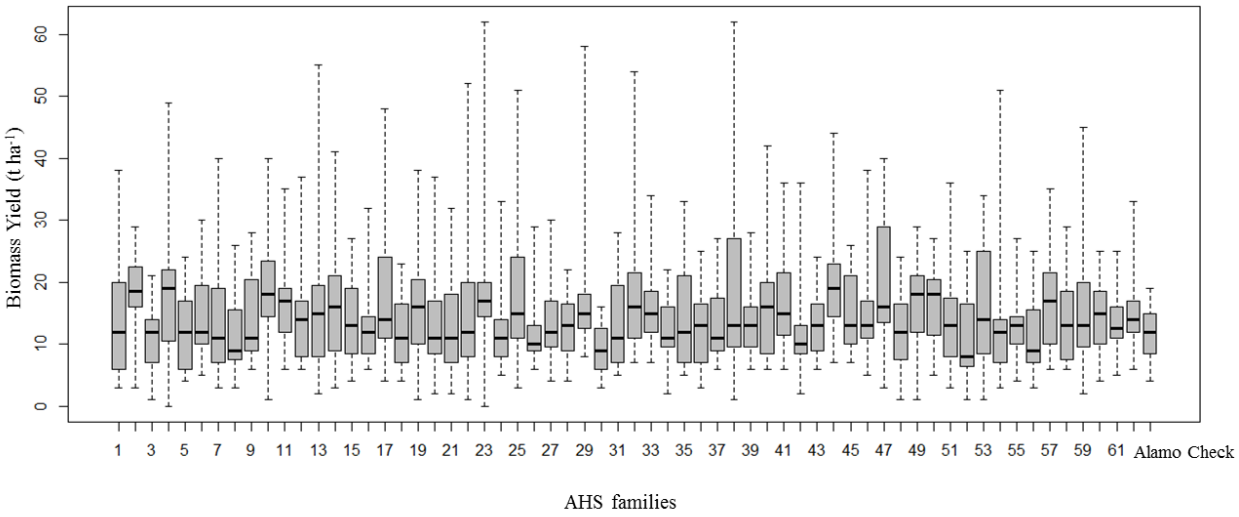

Figure 2A.1. Variation in biomass yield among and within the ‘Alamo’ half-sib (AHS) families across Knoxville and Crossville locations in 2013.

APPENDIX 2B

CHAPTER 2 SUPPLEMENTAL TABLES

Table 2B.1. Mean of 'Alamo' half-sib families (AHS) biomass yield and other agronomic traits across two years and two locations.

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11-A1	2.69	4	223	3	3	89
11-A3	3.55	5	233	4	3	88
11A-12	4.00	4	233	3	3	87
11A-13	3.29	4	225	3	4	89
11A-16	2.81	4	223	3	2	89
11A-23	4.17	4	241	3	4	88
11A-24	3.46	4	233	4	4	88
11A-27	3.22	4	229	3	4	89
11A-29	4.21	4	249	4	4	88
11A-30	3.63	4	228	4	3	88
11A-32	3.73	5	238	3	4	88
11A-33	3.45	4	244	3	3	88
11A-34	3.30	4	221	3	5	88
11A-35	3.98	4	235	3	4	88
11A-36	2.86	4	231	4	3	89
11A-38	3.33	4	218	3	3	88
11A-39	3.63	5	234	4	4	89
11A-40	2.90	4	228	3	3	88
11A-41	4.38	5	228	4	4	88
11A-42	4.04	4	243	3	5	88
11A-45	4.53	4	236	4	4	89
11A-47	2.96	4	222	3	3	89
11A-49	3.90	4	237	4	4	88
11A-51	3.66	4	242	3	3	88
11A-52	4.67	4	252	4	4	89
11A-53	2.73	4	237	3	3	88
11A-54	4.03	4	242	4	3	88
11A-56	3.48	4	251	3	4	88
11A-58	3.33	4	237	4	4	89
11A-59	2.79	3	-	3	3	88
11A-60	3.50	4	240	4	4	88
11A-61	3.94	5	242	4	4	89
11A-62	3.82	5	230	3	4	88

Table 2B.1. (continued).

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11A-63	3.53	4	243	3	3	88
11A-64	3.06	4	232	3	3	88
11A-66	2.88	4	-	4	3	89
11A-67	3.40	5	229	3	4	89
11A-68	2.89	4	239	4	3	89
11A-70	3.44	5	233	4	4	88
11A-71	3.25	5	243	4	4	87
11A-72	4.17	5	232	4	4	89
11A-73	3.29	4	221	4	4	90
11A-74	3.98	4	234	3	5	89
11A-75	4.28	5	235	4	4	89
11A-76	3.01	5	226	3	3	88
11A-77	3.36	4	248	3	3	88
11A-78	3.71	5	243	4	5	89
11A-79	3.40	4	234	4	4	89
11A-80	3.32	5	221	3	5	89
11A-81	4.22	5	234	4	4	88
11A-82	3.57	5	240	4	4	88
11A-83	3.72	4	242	4	4	89
11A-84	4.02	5	235	4	4	88
11A-87	2.79	4	238	3	3	89
11A-88	4.15	4	241	4	4	88
11A-89	4.18	5	256	4	5	89
11A-90	4.25	5	235	4	5	89
11A-91	3.40	4	245	4	2	89
11A-92	3.93	5	252	4	4	89
11A-93	3.97	4	232	3	4	89
11A-94	3.90	5	229	3	3	88
11A-95	3.56	5	236	3	5	89
Alamo	3.82	5	241	3	4	89
LSD	0.81***	1*	23	1	1***	1

Table 2B.2. Mean of ‘Alamo’ half-sib families (AHS) biomass yield and other agronomic traits at Knoxville location.

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11-A1	2.21	4	234	3	3	78
11-A3	2.51	4	238	4	3	78
11A-12	3.14	4	235	3	3	77
11A-13	2.95	4	222	3	4	78
11A-16	2.49	4	225	3	2	78
11A-23	3.93	4	249	3	4	78
11A-24	3.37	5	238	4	4	78
11A-27	2.83	4	234	4	4	79
11A-29	3.63	3	250	3	4	77
11A-30	3.35	4	219	4	3	77
11A-32	3.62	4	248	4	4	78
11A-33	2.27	4	240	3	3	77
11A-34	3.76	5	218	4	5	77
11A-35	3.71	4	229	3	4	77
11A-36	2.90	3	242	4	3	78
11A-38	2.95	5	214	4	3	78
11A-39	2.98	5	233	4	4	78
11A-40	2.76	4	229	3	3	77
11A-41	3.91	5	234	4	4	78
11A-42	4.08	4	244	3	5	78
11A-45	4.09	4	234	3	4	79
11A-47	2.39	4	225	4	3	79
11A-49	3.67	4	248	4	4	79
11A-51	3.16	4	240	3	3	78
11A-52	4.58	4	253	4	4	79
11A-53	2.77	3	243	3	3	78
11A-54	3.63	4	250	4	3	78
11A-56	3.30	4	266	4	4	78
11A-58	2.97	4	248	4	4	78
11A-59	2.66	3	223	3	3	78
11A-60	3.02	4	244	4	4	78
11A-61	4.21	5	252	4	4	78
11A-62	3.52	4	231	4	4	77

Table 2B.2. (continued).

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11A-63	3.10	4	256	3	3	77
11A-64	2.85	4	239	3	3	77
11A-66	2.42	4	249	3	3	78
11A-67	3.46	4	234	4	4	79
11A-68	2.60	4	239	4	3	78
11A-70	2.72	5	231	4	4	77
11A-71	3.29	5	247	4	4	77
11A-72	3.77	5	226	4	4	79
11A-73	3.42	4	226	4	4	79
11A-74	4.00	3	245	3	5	79
11A-75	3.75	5	238	4	4	78
11A-76	2.80	5	219	4	3	78
11A-77	2.71	4	253	3	3	78
11A-78	3.68	5	249	4	5	80
11A-79	3.22	4	247	4	4	79
11A-80	3.37	4	222	4	5	78
11A-81	3.61	5	226	4	4	78
11A-82	3.22	5	253	4	4	78
11A-83	3.31	4	252	4	4	79
11A-84	3.33	5	232	4	4	77
11A-87	2.55	4	236	4	3	78
11A-88	3.58	4	245	4	4	78
11A-89	3.71	4	269	4	5	79
11A-90	4.07	5	238	4	5	79
11A-91	2.65	4	248	4	2	78
11A-92	3.28	5	262	4	4	78
11A-93	3.49	4	239	3	4	79
11A-94	3.42	5	219	4	3	77
11A-95	3.17	5	243	4	5	78
Alamo	3.30	5	235	4	4	78
LSD	1.16	2	33	1	1***	2

Table 2B.3. Mean of ‘Alamo’ half-sib families (AHS) biomass yield and other agronomic traits at Crossville location.

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11-A1	3.18	5	212	3	-	99
11-A3	4.60	5	228	3	-	99
11A-12	4.85	5	231	3	-	98
11A-13	3.64	5	228	3	-	100
11A-16	3.14	5	220	3	-	100
11A-23	4.41	5	234	4	-	99
11A-24	3.55	4	228	3	-	99
11A-27	3.62	4	225	3	-	99
11A-29	4.78	5	247	4	-	98
11A-30	3.91	5	237	4	-	99
11A-32	3.84	5	228	3	-	99
11A-33	4.62	5	249	3	-	100
11A-34	2.84	4	224	3	-	99
11A-35	4.24	5	242	3	-	99
11A-36	2.82	4	219	3	-	99
11A-38	3.72	3	223	3	-	99
11A-39	4.28	5	235	4	-	99
11A-40	3.03	4	227	4	-	100
11A-41	4.85	5	223	3	-	99
11A-42	4.00	4	243	3	-	99
11A-45	4.98	5	239	4	-	99
11A-47	3.54	4	220	3	-	99
11A-49	4.12	4	226	4	-	98
11A-51	4.17	5	244	4	-	98
11A-52	4.76	4	251	3	-	99
11A-53	2.70	4	232	3	-	99
11A-54	4.44	4	233	3	-	99
11A-56	3.67	4	237	3	-	99
11A-58	3.69	5	225	4	-	100
11A-59	2.93	3	-	3	-	99
11A-60	3.98	4	235	4	-	99
11A-61	3.66	5	232	4	-	100
11A-62	4.12	5	228	3	-	100

Table 2B.3. (continued).

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11A-63	3.96	4	231	3	-	99
11A-64	3.27	4	226	3	-	100
11A-66	3.35	4	-	4	-	100
11A-67	3.35	5	223	3	-	99
11A-68	3.18	5	239	3	-	100
11A-70	4.16	4	235	3	-	99
11A-71	3.21	5	238	3	-	98
11A-72	4.58	5	238	3	-	99
11A-73	3.16	4	217	3	-	100
11A-74	3.97	5	224	3	-	99
11A-75	4.82	5	232	4	-	99
11A-76	3.22	5	234	3	-	98
11A-77	4.00	5	243	4	-	99
11A-78	3.74	5	236	4	-	99
11A-79	3.59	4	220	4	-	99
11A-80	3.27	5	220	3	-	99
11A-81	4.83	5	243	4	-	99
11A-82	3.92	5	226	4	-	99
11A-83	4.14	4	232	4	-	99
11A-84	4.72	4	238	4	-	99
11A-87	3.03	4	240	3	-	101
11A-88	4.72	4	237	4	-	99
11A-89	4.65	6	243	4	-	99
11A-90	4.42	5	231	4	-	99
11A-91	4.16	4	242	3	-	100
11A-92	4.59	5	242	3	-	99
11A-93	4.46	4	225	4	-	99
11A-94	4.38	5	238	3	-	99
11A-95	3.94	5	228	3	-	99
Alamo	4.34	5	247	3	-	99
LSD	1.16	2	33	1	-	2

Table 2B.4. Mean of 'Alamo' half-sib families (AHS) biomass yield and other agronomic traits in 2013 from both locations.

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11-A1	2.33	5	225	4	3	-
11-A3	3.16	6	255	5	3	-
11A-12	3.40	5	245	4	3	-
11A-13	3.12	5	227	4	4	-
11A-16	2.70	5	226	4	2	-
11A-23	3.48	5	248	4	4	-
11A-24	3.30	5	246	4	4	-
11A-27	2.88	5	237	4	4	-
11A-29	3.62	5	261	4	4	-
11A-30	3.38	5	241	4	3	-
11A-32	3.51	5	257	4	4	-
11A-33	3.28	4	260	4	3	-
11A-34	3.03	5	238	4	5	-
11A-35	3.55	5	251	4	4	-
11A-36	2.60	4	245	4	3	-
11A-38	3.04	4	223	4	3	-
11A-39	3.26	6	248	5	4	-
11A-40	2.61	4	238	4	3	-
11A-41	3.92	6	231	4	4	-
11A-42	3.71	5	264	4	5	-
11A-45	4.01	5	235	4	4	-
11A-47	2.54	5	234	4	3	-
11A-49	3.22	5	243	4	4	-
11A-51	2.97	5	249	4	3	-
11A-52	3.99	5	264	4	4	-
11A-53	2.14	4	249	3	3	-
11A-54	3.12	5	236	4	3	-
11A-56	3.10	4	277	4	4	-
11A-58	3.18	5	233	4	4	-
11A-59	2.84	4	-	3	3	-
11A-60	3.44	5	240	4	4	-
11A-61	3.61	5	250	5	4	-
11A-62	3.32	6	238	4	4	-

Table 2B.4. (continued).

Family	Biomass Yield (kg plot^{-1})	Tillering Ability (score)	Plant Height (cm)	Stem Thickness (score)	Spring Vigor (score)	Spring Regrowth (Julian Calendar)
11A-63	3.21	4	254	4	3	-
11A-64	2.86	5	238	4	3	-
11A-66	2.67	4	-	4	3	-
11A-67	3.12	5	233	4	4	-
11A-68	2.61	6	248	5	3	-
11A-70	3.08	5	238	4	4	-
11A-71	3.12	5	249	5	4	-
11A-72	3.75	5	238	4	4	-
11A-73	2.90	5	238	4	4	-
11A-74	4.04	5	235	4	5	-
11A-75	3.48	5	245	5	4	-
11A-76	2.65	6	239	4	3	-
11A-77	2.93	4	252	4	3	-
11A-78	3.74	6	253	5	5	-
11A-79	2.87	4	245	4	4	-
11A-80	3.35	6	242	4	5	-
11A-81	3.63	5	241	5	4	-
11A-82	3.25	5	246	4	4	-
11A-83	3.12	4	261	4	4	-
11A-84	3.92	5	249	5	4	-
11A-87	2.30	5	243	4	3	-
11A-88	3.64	5	248	5	4	-
11A-89	3.62	5	262	4	5	-
11A-90	4.07	5	238	5	5	-
11A-91	3.16	4	252	4	2	-
11A-92	3.29	5	255	5	4	-
11A-93	3.67	5	239	4	4	-
11A-94	3.23	6	233	4	3	-
11A-95	3.33	5	251	4	5	-
Alamo	3.28	5	249	4	4	-
LSD	0.93**	1	24	1	1***	-

CHAPTER 3

IDENTIFICATION OF QUANTITATIVE TRAIT LOCI (QTL) ASSOCIATED WITH BIOMASS YIELD AND ESTABLISHMENT RELATED TRAITS USING A NESTED ASSOCIATION MAPPING (NAM) POPULATION OF LOWLAND SWITCHGRASS

ABSTRACT

Switchgrass is a model herbaceous crop selected for the bioenergy feedstock development in the United States. Biomass yields of available cultivars are inadequate and its improvement is important for the profitability of switchgrass as a bioenergy crop. The long breeding cycles in switchgrass limit its rapid genetic improvement and thus, methods that can overcome this limitation are warranted. Dissection of genetic factors or quantitative trait loci (QTL) associated with complex traits and development of associated molecular markers could accelerate switchgrass improvement for biofuel production. The goals of this study are (i) to evaluate the genetic variation in switchgrass (ii) to detect and map QTLs related to biomass yield, seed weight, and germination, and (iii) to identify associated molecular markers for use in marker-assisted breeding of switchgrass. A Nested Association Mapping (NAM) population established in Ardmore, OK and Knoxville, TN was utilized in this study. Results showed significant variations among NAM families for biomass yield, seed weight, and germination ($P<0.05$). Significant variation among genotypes within families was observed in biomass yields ($P<0.05$). The top-performing NAM families were observed from the crosses between AP13 and the nested parents that were improved cultivars derived from Alamo and Kanlow. A total of 785 significant markers for biomass yield were detected ($P<0.05$) of which 176 were for seed weight and 131 for germination. Composite interval mapping revealed 21 QTLs for biomass yield across locations in 2 years the highest LOD score of 7.6 detected in chromosome Ia that explained a 12.5% of the phenotypic variation. Two QTLs located in chromosome VIIIb were detected for seed weight with LOD scores of 2.7 and 2.8 and explained 5.2% and 4.6% phenotypic variation. One QTL for germination located on chromosomes IXa was detected with a LOD score 3.5 and explained 5.2% of the phenotypic

variation. SNP markers closely linked to important QTL were also identified that could be used for marker-assisted breeding.

Abbreviations: SNP Single Nucleotide Polymorphism, QTL Quantitative Trait Loci, SMA Single Marker Analysis, IM Interval Mapping, CIM Composite Interval Mapping

INTRODUCTION

Switchgrass (*Panicum virgatum* L.) has been regarded as the model herbaceous crop for the bioenergy feedstock production in the United States (McLaughlin *et al*., 1999; McLaughlin, 1992; Wright and Turhollow, 2010). Switchgrass is a warm-season C4 grass with perennial growth habit. It is used for forage, soil conservation, and bioenergy production (Casler, 2012; McLaughlin and Walsh, 1998). Aside from its high biomass yield potential, switchgrass was chosen due to its ability to grow well in marginal lands with less input, sequester a large amount of atmospheric CO_2 into the soil, and promote wildlife diversity (Blanco-Canqui, 2010; Ma *et al*., 2000).

Native populations of switchgrass are known for high genetic diversity. Broadly, switchgrass populations are classified into two ecotypes, lowland and upland. The two ecotypes are also characterized as two cytoplasm types, "L" for lowland and "U" for upland, wherein a deletion of 49 nucleotides in the non-coding region of chloroplastic trnL DNA is specific to lowland cytotypes (Hultquist *et al*., 1996; Missaoui *et al*., 2006; Vogel, 2004). Lowland ecotypes are adapted to wetter environments in the southern regions while upland ecotypes are adapted to relatively dry and colder environments in the north (Lowry *et al*., 2014; Moser and Vogel, 1995; Vogel, 2004). Lowland switchgrass, due to its high biomass yield potential would be an excellent candidate for genetic improvement targeting bioenergy feedstock use.

Switchgrass is a relatively new species with respect to its cultivar breeding history. Most of the available cultivars are the result of direct selection from native germplasm and only a few cultivars released involved some systematic breeding (Casler, 2012). Biomass yield of currently available cultivars is inadequate, and its improvement is critically important for the profitability of switchgrass as a bioenergy crop (Perrin *et al*., 2008; Schmer *et al*., 2008). A common method of

improving biomass yield is through genetic improvement and development of new cultivars (Bartley *et al*., 2013).

Switchgrass is a cross-pollinated species and its cultivar breeding methodology includes recurrent phenotypic selection with some restrictions (Burton, 1982) and genotypic selection based on half-sib or full-sib progeny-performance (Casler and Brummer, 2008). It has also been recognized through years of breeding efforts that the rate of genetic gain in yield of perennial grasses is slow compared to the annual grain crops (Casler and Brummer, 2008; Humphreys, 2005). In bahiagrass, the recurrent restricted phenotypic selection resulted in a 2% to 6% gain in forage yield per cycle of selection Burton (1982). Genotypic selection based on half-sib or full-sib family performance could improve selection gain (Brummer and Casler, 2009; Casler and Brummer, 2008; Vogel and Burson, 2004). However, conventional breeding efforts take longer time as switchgrass' first-year yield shows poor correlation with the plant performance in the succeeding years, and breeding populations require evaluating at least two years before making any selection. This long selection cycle limits rapid genetic improvement in switchgrass. Thus, an efficient system that can overcome this challenge is warranted.

Despite the resilience of switchgrass plants to varying environmental factors, the species is known to possess high seed dormancy and small-sized seeds that hinder successful establishment (Evers and Parsons, 2003; Loch *et al*., 2004; Mitchell and Vogel, 2012). A study by Duclos *et al*. (2013) revealed that switchgrass seed dormancy is mainly caused by coat-imposed dormancy. However, the presence of embryo dormancy cannot be overruled as Haynes *et al*. (1997) demonstrated an increase germination using coat-related dormancy-breaking methods, acid treatment and sodium hypochlorite, in combination with embryo dormancy-breaking method, wet chilling. Similarly, germination is also found to be influenced by seed size (Boe and Johnson,

1987; Green and Hansen, 1969; Smart and Moser, 1999). Switchgrass has relatively small seed size (Casler, 2012) and has an average of 850 seeds g^{-1} (Sanderson *et al*., 2012) or approximately 1 mg $seed^{-1}$. A laboratory study on several lowland and upland cultivars of switchgrass showed that switchgrass emergence was improved with increased seed size (Aiken and Springer, 1995).

Both high seed dormancy and small seed size may result in poor stand which reduces yield per unit area (Buhler *et al*., 1998). Most of the past studies on stand establishment have focused on approaches to improve weed management during establishment instead of breeding for improved germination and seed size (Casler, 2012). In most field crops, seed germination and seed weight have been successfully improved through selection (Akinyosoye *et al*., 2014, Finch-Savage and Bassel, 2016; Yamane *et al*., 2018). Significant variation in seed germination and seed size in switchgrass demonstrates the potential for their improvement (Finch-Savage and Bassel, 2016). As with biomass yield, seed germination and seed weight are complex traits and are highly influenced by the environment (Basnet *et al*., 2015; Boe, 2003), thus improvement of these traits using conventional procedures may take several cycles of selection. Dissection of genetic factors or quantitative trait loci (QTL) associated with these complex traits and development of associated molecular markers and deployment of marker-assisted selection could accelerate switchgrass improvement for biofuel production.

Most of the QTL detection studies in the past have utilized biparental mapping populations (Hackett *et al*., 2013; Li *et al*., 2008; Liu *et al*., 2006; Santra *et al*., 2008; Xu and Mackill, 1996). In switchgrass, Lowry *et al*. (2015) used a mapping population derived from Alamo × Kanlow genotypes and identified 27 significant QTLs, 2 of which are QTLs for biomass yield with 10% and 12% of the phenotypic variation explained and showed positive additive effects. Using a cross between lowland AP13 (Alamo genotype) and upland VS16 (Summer genotype), Serba *et al*.

(2015) identified 34 QTLs related to biomass yield with 3.5 to 15.3% of the phenotypic variation explained and 38 QTLs related to plant height with 4.3 to 17.4% phenotypic variation explained which showed mostly negative additive effects. Chang *et al*. (2016) identified 20 QTLs for tillering-related traits with 5.7 to 17.8% of the phenotypic variation explained from a biparental cross between a northern lowland genotype, NL 94, and a southern lowland genotype, SL 93. However, biparental populations allow limited recombination events and the genetic variation is limited to the two parents, thus limiting the scope of QTL mapping (Xu *et al*., 2017).

Multiparent mapping population is therefore warranted to overcome the limitation of using biparental mapping population since the multiple parents will capture greater allelic diversity for high-resolution QTL mapping (Xu *et al*., 2017). The availability of high throughput genotyping and reduced cost have made it possible to dissect complex traits using multi-parent populations thus improving both the power and the scope (Voss-Fels and Snowdon, 2016). A Nested Association Mapping (NAM) population study generated from 25 diverse germplasm founders and a common parent was first demonstrated in maize to dissect complex genetics of important traits since NAM combines the advantages of both linkage and association mapping (McMullen *et al*., 2009; Yu *et al*., 2008). Both maize and switchgrass are cross-pollinated species, thus the approach used in maize can also be applicable to switchgrass. In this study, we used a NAM population of lowland switchgrass to dissect the genetics of important traits.

The objectives of this study were: (i) to evaluate the genetic variation in switchgrass for biomass yield, and establishment related seed traits, (ii) to detect and map QTLs related to biomass yield, seed weight, and seed germination, and (iii) to identify molecular markers associated with these QTLs for use in marker-assisted breeding of switchgrass.

MATERIALS AND METHODS

Plant Materials

A Nested Association Mapping (NAM) population was developed at the Noble Research Institute, LLC in Ardmore, Oklahoma by crossing fifteen selected genotypes to a common parent, 'AP13,' a genotype selected from a lowland Alamo population. The 15 genotypes used for the development of NAM population were selected from a diverse germplasm collection and represented a wide diversity in morphological traits (Table 3A.1). AP13 was used as a common parent because it is widely used in switchgrass genomics research including whole genome sequencing at the DOE Joint Genomics Institute. For each resulting AP13 × genotype cross family, 10 F_1 plants were produced and chain-crossed to generate recombinant ten chain-cross families. Twenty random genotypes for each chain-cross family were included in the mapping population. The 15 parent genotypes initially crossed with AP13 will be hereafter referred to as 'nested parents'. Similarly, F_1 genotypes selected from each of the AP13 × genotypes crosses and used in the chain cross will be hereafter referred to as 'chain-cross parents'.

All the planting materials were generated in the greenhouse of Noble Research Institute, LLC, Ardmore, OK. Initially, we planned to generate NAM population of 2,000 genotypes produced from 10 AP13 × genotype crosses, i.e., 10 AP13 × genotype crosses × 10 chain cross families derived from 10 F_1 per AP13 × genotype cross × 20 genotypes per chain-cross family (Figure 1). However, some chain-cross families did not produce the required number of seedlings, thus varying number of chain-cross families from the other 5 AP13 × genotype crosses were included to achieve the target population size. Including AP13, nested parents, chain-cross parents, and Alamo checks, the mapping population had a total of 2,350 genotypes (i.e., 2000 progenies

from 15 families, 30 ramets of AP13, three copies of nested parents, two copies of chain-cross parents, and 5 clonal copies of an Alamo check). All the seedlings and clonal propagule of parental genotypes were clonally propagated for evaluation in replicated trials in two locations: Ardmore, OK and Knoxville, TN.

Field Experiment

The NAM field nursery was established at two locations, Noble Research Institute, LLC at Ardmore, OK (34°13'13.5"N 97°12'15.3"W) and Plant Science Unit (35°57'16.5"N 83°55'46.1"W) of East Tennessee Research and Education Center (ETREC), Knoxville, TN. The soil type at the Ardmore site is a Wilson silt loam soil (fine, smectitic, thermic Oxyaquic Vertic Haplustalfs. The soil type at the Knoxville site is a Shady loam soil (fine-loamy, mixed, subactive, thermic Typic Hapludults). The experiment was established in an alpha lattice with 0.90 m × 0.90 m plant spacing in Knoxville and 1 m × 1 m plant spacing in Noble and two replications. Each replication was planted in a block of 47 rows × 50 plants. Due to the insufficient number of ramets produced, only one replication was established in 2013 while the other replication was established in 2014. The ramets were transplanted in June 2013 for replication 1 and July 2014 for replication 2 in Knoxville and August 2013 for replication 1 and July 2014 in Ardmore. Fertilizer was not applied during the establishment year. In each spring of the post-establishment years, 60 kg N ha^{-1} was applied. Pre-emergence herbicides, Prowl H_2O (Pendimethalin, BASF Corporation, Research Triangle Park, NC) at 3.31 L ha^{-1} and Dual II Magnum (Metolachlor, Syngenta, Crop Protection, Inc. Greensboro, NC) at 2.84 L ha^{-1} were applied during spring of each year. About 60 days after transplanting, post-emergence herbicide, 2,4-D at 2.37 L ha^{-1} with surfactant at 1.18 L ha^{-1} was applied.

Phenotypic Data Collection

Biomass yield was recorded in the Fall of 2014 (i.e., year-2 of rep 1) and 2015 (i.e., year-3 of rep1 and year-2 of rep2). About 200 g biomass was sampled from each plot for moisture content. Samples were dried in a batch oven (Wisconsin Oven Corporation, East Troy, WI, USA) for 48 hours at 49^{o}C. Sample fresh weight and dry weight were obtained, moisture content at harvest was determined, and was used to compute the dry matter yield of the corresponding entries in each location.

In the Fall of 2015, five panicles were harvested from each plant from the 2013 planted block from both test sites. Seed samples were collected in the Fall of 2015 (year-3 of rep1) from Ardmore, OK and Knoxville, TN. The seed was threshed and cleaned in the Spring of 2016. Seed weight was recorded from 100 seeds. After weighing, the seeds were subjected to germination. The germination test started in July 2016 and ended in August 2016. No seed treatment was done prior to germination. Seeds were germinated in 100 mm × 15 mm petri-plates (Fisher Scientific Co. LLC**,** Pittsburgh, PA) lined with a layer of wet filter paper. Petri-plates with seeds were stacked in a box, with two filter paper-lined petri-plates without seeds placed at the bottom and at the top of each stack. The seed stacks were then stored at room temperature (25°C) and kept in the dark by closing the box. After fourteen days, the number of germinated seedlings was recorded.

Genotyping

Genotyping of the NAM population was carried out at Shawn Kaeppler's laboratory at the University of Wisconsin. The NAM population including chain-cross and nested parents, and common parent, AP13 were genotyped using exome-capture sequencing protocol as described in Evans *et al*. (2014). The resulting exome sequences were then mapped to the switchgrass reference

sequence, AP13 (*P. virgatum* v1.1, DOE-Joint Genomic Institute, http://www.phytozome.net/panicumvirgatum) to identify SNPs. A total of 2,684 SNPs were identified and were used to construct a linkage map using JoinMap (v4.1) software (Figure 3A.3). Genotyping data based on single nucleotide polymorphisms (SNP) was received from Noble Research Institute, LLC for QTL analysis.

Data Analysis

Phenotypic Data

Data on biomass yield, seed weight, and seed germination were analyzed using MIXED model in SAS (SAS Institute, 2013). Location and year were used as fixed and family and genotypes within the family were used as random factors in the model. Least squares means across locations were obtained and their statistical differences were determined based on Fisher's protected least significant difference ($P<0.05$). Mean values were used for QTL mapping analysis.

QTL Analysis

The QTL analysis for biomass yield, 100 seed weight, and germination was conducted using WinQTL Cartographer version 2.5 (Wang *et al*., 2012). Marker-trait associations were analyzed using Single Marker Analysis (SMA) followed by simple Interval Mapping (IM). QTLs detected were then subjected to Composite Interval Mapping (CIM). CIM was performed through forward and backward stepwise regressions with a $P< 0.05$ threshold for automatic cofactor selection. Other CIM parameters include a window size of 10, and a 1.0 cM chromosome walking speed. QTL with a logarithm of odds (LOD) score of 2.5 and above were reported as a QTL. The SNP closely associated with the QTLs were also determined from the CIM analysis. To check if the SNPs are associated with known genes, nearby sequences were obtained using Geneious 11.1.2

software (Kearse *et al*., 2012) and similarity search was done using NCBI BLASTn (Altschul *et al*., 1990).

RESULTS AND DISCUSSION

Phenotypic Variation

Biomass Yield

The NAM families did not differ in their mean biomass yield ($P>0.05$) across two locations (Knoxville, TN and Ardmore, OK) in 2 years (Table 3A.2). There was a significant variation among genotypes within families ($P<0.01$). Biomass yield was also influenced by family × location and genotype(family) × location interactions ($P<0.05$). However, family and genotype (family) interactions with year were not evident. The fixed effects of location and location × year were not significant ($P>0.05$) but year effect was significant ($P<0.01$). Analysis by location showed that biomass yield varied among ($P<0.05$) family in both locations but genotype within family only differed in biomass yield ($P<0.01$) in Ardmore. Year effect was significant ($P<0.01$) in both locations.

The significant variation in biomass yield among the NAM families and genotypes within the NAM families suggested the potential for yield improvement through recurrent selection. Year effect was evident across locations and within each location which reflects the fact that full biomass yield potential in switchgrass can only be realized during the third production year since perennial grasses invest significant amount of resources in the root development during their initial years of growth (McLaughlin *et al*., 1999).

The mean for biomass yield and their range across locations and years were presented in Table 3A.3. The overall NAM mean biomass yield was 0.81 kg plant^{-1} and it ranged from 0.04 kg to 1.97 kg plant^{-1}. The overall mean biomass yield of chain cross parents was 1.12 kg plant^{-1} and it ranged from 0.22 kg plant^{-1} to 2.26 kg plant^{-1}. The 15 nested parents had a mean biomass yield

of 0.98 kg plant^{-1} with values from 0.24 kg plant^{-1} to 1.90 kg plant^{-1}. AP13, the reference genotype, had a mean biomass yield of 0.76 kg plant^{-1} and Alamo had a mean biomass yield of 1.44 kg plant^{-1}. Mean separation revealed significant difference ($P<0.05$) of NAM with chain cross parents and Alamo. No significant difference was observed when NAM mean biomass yield was compared with the nested parents and AP13.

The nested parents and AP13 both exhibited low biomass yield. The chain-cross parents, on the other hand, showed a high biomass yield. This suggests heterosis exhibited by the chain cross parents since they were derived from AP13 × nested parents crosses. However, the NAM genotypes exhibited lower yield as compared to the hybrid chain cross parents. This could be attributed to the loss of heterosis due to sib mating.

The top 5 performing NAM families in Knoxville and Ardmore based on their biomass yield were from the crosses between AP13 and the nested parents EG 1104-1, EG 1101-1, EG 1101-2, and EG 1102-2. Such performance could reflect the fact that these four nested parent genotypes were selected from improved cultivars, EG1104, EG1101, and EG1103 (Bouton and Wood, 2010, Bouton and Wood, 2012). This was also reflected by the superior performance of these four genotypes among the 15 nested parents used in NAM development. The AP13 × PI 414065 cross also performed well in terms of biomass yield. PI 414065 was chosen as one of the diverse NAM parents due to its high biomass yield (Saha *et al*., 2016).

The frequency distribution of biomass yield from Knoxville and Ardmore in 2015 showed a relatively normally distributed data of the NAM families (Figure 3A.2a). AP13, the female parent of the NAM population, appeared to be in the middle of the distribution while Alamo, the cultivar where AP13 was derived, appeared to be in the higher end of the distribution.

Seed Weight

In terms of the seed traits observed, NAM families differed in their seed weight ($P<0.05$) (Table 3A.4). Variation among genotypes within the family was not significant ($P<0.05$). The effect of location was significant ($P<0.05$).

The mean for seed weight and the range across two locations in 2015 are presented in Table 3A.5. The overall NAM mean seed weight was 56.06 mg 100 seed^{-1} and it ranged from 14.30 mg to 225.75 mg 100 seed^{-1}. The overall mean seed weight for chain cross parents was 51.01 mg 100 seed^{-1} and it ranged from 16.40 mg to 130.40 mg 100 seed^{-1}. The 15 nested parents had a mean seed weight of 46.02 mg 100 seed^{-1} with values from 18.90 mg to 96.00 mg 100 seed^{-1}. AP13, the reference genotype, had a mean seed weight of 56.60 mg 100 seed^{-1} and Alamo had a mean seed weight of 43.04 mg 100 seed^{-1}. No significant differences ($P>0.05$) were observed when NAM mean seed weight was compared with the chain cross parents, nested parents, AP13, and Alamo. The frequency distribution of seed weight showed that both Alamo and AP13 were in the middle part of the distribution but with AP13 slightly higher than Alamo (Figure 3A.2b).

In terms of seed weight, the top performing NAM families were from the crosses between AP13 and the nested parents EG 1101-2, PI 421521-1, PI 315723-1, PI 442535, and EG 1104-1. As stated before, nested parents EG 1101-2 and EG 1104-1 were selections from improved cultivars that could have explained higher seed weight of crosses involving these parents. PI 421521-1, PI 315723-1, and PI 442535 were genotypes selected from plant introductions based on early plant vigor, plant height, and rust tolerance. Although these traits are not directly related to seed weight, it is possible that they could have contributed to better seed development.

Seed Germination

The NAM families differed in their germination ($P<0.05$, Table 3A.4.). Variation among genotypes within family did not differ in germination ($P>0.05$). The effect of location was not important. The overall NAM mean seed germination was 77% and it ranged from 5 to 100% (Table 3A.5). The overall mean germination for the chain cross parents 80% and it ranged from 10 to 100%. The 15 nested parents had a mean seed germination of 81% with values from 16 to 100%. AP13, the reference genotype, had a mean seed germination of 66% while Alamo had a mean seed germination of 83%. No significant differences ($P>0.05$) were observed when NAM mean seed weight was compared with the chain cross parents, nested parents, AP13, and Alamo. The frequency distribution for germination showed that AP13, the female parent of the NAM population, appeared to be in the lower end of the as compared to Alamo (Figure 3A.2c).

The top-performing NAM families for seed germination were from the crosses between AP13 and the diverse parents EG 1101-1, EG 1102-1, PI 442535, EG 1102-2, and EG 1104-2. As observed from biomass yield and seed weight, the progenies from the crosses with improved genotypes performed well.

QTL Analysis

Single Marker Analysis

Single marker analysis (SMA) was performed using simple linear regression, where individual markers were used as independent variables and biomass yield, seed weight, and seed germination were used as dependent variables. SMA detected 785 significant markers ($P<0.05$) for biomass yield. There were 450 significant markers at $P<0.01$, 205 markers at $P<0.001$, and 95

markers at P<0.0001 (Table 3B.1). A total of 176 significant markers (P<0.05) were detected for seed weight and 17 markers were significant at P<0.01. For seed germination, there were 131 significant markers detected (P<0.05) and 23 markers were significant at P<0.01. Single marker analysis was done as a screening procedure to easily detect QTLs since it is considered the simplest method of QTL detection (Collard *et al*., 2005). However, interval mapping methods should follow to confirm SMA results.

Interval Mapping

A simple Interval Mapping (IM) was performed to quickly screen the data set for the presence of QTLs. For biomass yield, IM detected 34 QTLs interspersed in all the chromosomes. In terms of seed weight and germination, there were no significant QTLs detected. These results were further confirmed through Composite Interval Mapping (CIM).

Composite Interval Mapping confirmed several QTLs that were detected using IM. These QTLs can be seen interspersed throughout the chromosomes as seen in Figure 4. Twenty-one of the 34 biomass QTLs detected by IM were also identified by CIM (Table 3A.6; Figure 3A.4). Biomass QTL with the highest LOD score of 7.6 was detected in chromosome Ia that explained a 12.5% of the phenotypic variation and showed a 0.10 kg plant^{-1} additive effect (Figure 3A.5a). The nearest SNP to this QTL was c1a_70541448. The biomass QTL with the second highest LOD score was 5.2, located in chromosome Va, explained 7.7% of the phenotypic variation and showed 0.05 kg plant^{-1} additive effect. The nearest SNP to this QTL was c5a_3277815.

Biomass yield QTLs specific to Knoxville location were identified. There were 2 QTLs detected by CIM. The QTL with LOD score of 4.2 was in chromosome IXa (Figure 3A.5b). This QTL explained 3.4% of the phenotypic variation and showed a 0.04 kg plant^{-1} additive effect. The

nearest SNP to this QTL was c9a_53017202. The second QTL was found to be located on chromosome IIb with a LOD of 3.2 and explained a 3.5% of the phenotypic variation. The QTL has an additive effect of -0.04 kg plant^{-1} and the SNP it was associated with was c2b_40987348.

Biomass yield QTLs specific to Ardmore were also identified. There were 14 QTLs detected by CIM. The QTL with LOD score of 12.3 was in chromosome VIIIa (Figure 3A.5c). This QTL explained 11.6% of the phenotypic variation and showed a 0.12 kg plant^{-1} additive effect. The nearest SNP to this QTL was c8a_18503891. The second QTL was found to be located on chromosome Va with a LOD of 8.6 and explained a 10.2% of the phenotypic variation. The QTL has an additive effect of 0.09 kg plant^{-1} and the SNP it was associated with was c5a_16084879.

Composite interval mapping (CIM) analysis for seed weight detected 2 QTLs (Table 3A.6; Figure 3A.6 and 4A.7a). These QTLs were located in chromosome VIIIb with LOD scores of 2.7 and 2.8 and explained 5.2% and 4.6% phenotypic variation and had a -4.53 mg 100 seed^{-1} and 3.69 mg 100 seed^{-1} additive effects, respectively. These effects are comparable to the range of effects from seed weight QTL studies in other crops (Boyles *et al*., 2017; Pan *et al*., 2017; Tang *et al*., 2013). The nearest SNP to the first QTL was c8b_8355808 and has a significant association with seed weight as observed in SMA. The nearest SNP to the second QTL was c8b_14530598 and it also has a significant association with seed weight as observed from SMA.

For seed germination, CIM detected only one QTL (Table 3A.6; Figure 3A.6 and 3A.7b). The QTL was located on chromosomes IXa with a LOD score of 3.5, explained 5.2% of the phenotypic variation and contributed 3% additive effect. These effects are also comparable to the range of effects from germination QTL studies in other crops (Hongjun *et al*., 2017, Wan *et al*.,

2005). The nearest SNP to this QTL was c9a_73835923 which showed a significant association with seed germination as observed from SMA.

Using the AP13 reference sequence (*P. virgatum* v1.1, DOE-Joint Genomic Institute, http://www.phytozome.net/panicumvirgatum) and physical location of the SNPs associated with detected QTLs, nearby sequences were obtained using Geneious 11.1.2 software (Kearse *et al*., 2012). A quick similarity search was done using NCBI BLAST (Altschul *et al*., 1990) (Table 3A.7). The top 3 biomass yield QTLs were found to have similarity (78 to 94% identity; 0 to 3E-152 E-values) with catalases (Yang and Poovaiah, 2002), RNA helicases (Barak *et al*., 2014), and WRKY transcription factors, respectively, which are indirectly related to biomass yield since these genes are related to stress responses. (Jiang *et al*., 2017). Seed weight QTL from chromosome VIIIb have similarity with polygalacturonase (85 to 89% identity; 5E-11 to 5E-16 E-value) which is related to seed development (Hadfield and Bennett, 1998). The germination QTL from chromosome IXa have similarity with zinc finger proteins (78 to 89% identity; 2E-64 to 9E-178 E-value) which were found to have a role in germination (Baek *et al*., 2015; Park *et al*., 2017). It is noteworthy that most sequences were found to have similarities with species *Setaria italica* and *Sorghum bicolor*. This could be due to the close phylogenetic relationship of switchgrass with *Sorghum* and *Setaria* (Daverdin *et al*., 2015; Okada *et al*., 2010).

The current study was able to identify several QTLs for biomass yield, seed weight, and germination and find SNPs closely linked with these QTL. These associated SNPs will be useful in marker-assisted breeding. However, these QTLs should be validated using advanced independent populations to check their stability in different genetic backgrounds.

Although several QTL studies have been conducted for biomass yield, our research utilized a NAM population as compared to previous studies that are mostly biparental mapping populations

(Lowry *et al*., 2015; Serba *et al*., 2015). We were able to detect more QTLs with greater positive additive effects as compared to previous studies, thus elucidating the advantage of using more parents in mapping population development. Also, to our knowledge, this is the first QTL study for seed weight and seed germination in switchgrass and provided insights into the presence of seed weight and germination QTLs. Once validated, screening switchgrass populations for the desired trait will be possible using the associated markers that will hasten cultivar improvement in switchgrass breeding.

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APPENDIX 3A

CHAPTER 3 TABLES AND FIGURES

Table 3A.1. Parental genotypes used in Nested Association Mapping (NAM) population development.

Accession	Origin	Description	Desired Traits
PI 414065	Arkansas	BN-14668-65	High biomass yield
PI 442535	Belgium	156	Rust tolerant
PI 421521-1	Kansas	GRIN accession of Kanlow	Early plant vigor
PI 421521-2	Kansas	GRIN accession of Kanlow	High biomass yield
PI 315725	Mississippi	BN-14669-92	Seed retention
PI 315723-1	North Carolina	BN-8358-62	Plant height
PI 315723-2	North Carolina	BN-8358-62	Seed retention
PI 315723-3	North Carolina	BN-8358-62	Early plant vigor
PI 422006	Texas	GRIN accession of Alamo	Plant height
EG 1101-1	Georgia	Improved variety derived from Alamo; derived by intercrossing 25 genotypes which originated from five clones that were selected based on half-sib progeny performance	Compact panicle
EG 1101-2	Georgia	Improved variety derived from Alamo; derived by intercrossing 25 genotypes which originated from five clones that were selected based on half-sib progeny performance	Early regrowth
EG 1102-1	Georgia	Improved variety derived from Kanlow; derived by intercrossing 25 genotypes which originated from five clones that were selected based on half-sib progeny performance	Late heading
EG 1102-2	Georgia	Improved variety derived from Kanlow; derived by intercrossing 25 genotypes which originated from five clones that were selected based on half-sib progeny performance	Rust tolerant
EG 1104-1	Georgia	Improved variety derived from crossing Alamo and Kanlow	Early regrowth
EG 1104-2	Georgia	Improved variety derived from crossing Alamo and Kanlow	Plant height

Table 3A.2. Components of variation of the Nested Association Mapping (NAM) population for biomass yield in Ardmore, OK, Knoxville, TN and across locations in two years (2014-2015).

Source of Variation	Biomass Yield (kg plant^{-1}) Knoxville	Ardmore	2 locations, 2 years
	Estimates of variance components		
Rep/Rep(Location	0.041	0.011	0.025
Family	0.004*	0.059*	0.016
Genotype (Family)	0.000	0.018***	0.020***
Family × Location	-	-	0.016*
Genotype(Family) × Location	-	-	0.028***
Genotype(Family) × Rep/Rep(Location)	0.168***	0.084***	-
Family × Year	0.001	0.015**	0.002
Genotype(Family) × Year	0.000	0.000	0.000
Family × Location × Year	-	-	0.005*
Genotype(Family) × Location × Year	-	-	0.000
Residual	0.130***	0.132***	0.221***
	Test of fixed effects (*F* values)		
Location	-	-	1.31
Year	931***	121***	303***
Location × Year	-	-	3.94

* Significant at P<0.05.
** Significant at P<0.01.
*** Significant at P<0.001.

Table 3A.3. Mean biomass yield and range of the Nested Association Mapping (NAM) population, Chain cross Parents, Nested Parents, AP13, and Alamo across two locations in two years (2014-2015).

	Biomass Yield (kg plant^{-1})		
	Maximum	Mean	Minimum
NAM	1.97	0.81[a]	0.04
Chain cross Parents	2.26	1.12[b]	0.22
Nested Parents	1.90	0.98[ab]	0.24
AP13	-	0.76[ab]	-
Alamo	-	1.44[ab]	-

Table 3A.4. Components of variation of the Nested Association Mapping (NAM) population for seed weight and germination across locations in 2015.

Source of Variation	Seed Weight[†]	Germination[‡]
	Estimates of variance components	
Family	33.52**	0.001*
Genotype(Family)	0.00	0.003
Residual	1008***	0.070***
	Test of fixed effects (*F* values)	
Location	5.53**	1.89

[†] seed weight = mg 100 seed^{-1}
[‡] germination = %
[*] Significant at P<0.05.
[**] Significant at P<0.01.
[***] Significant at P<0.001.

Table 3A.5. Mean and range of seed weight and germination of the Nested Association Mapping (NAM) population, Chain cross Parents, Nested Parents, AP13, and Alamo across two locations in 2015.

	Seed Weight (mg 100 seed^{-1})			Seed Germination (%)		
	Maximum	Mean	Minimum	Maximum	Mean	Minimum
NAM	225.75	56.06	14.30	100	77	5
Chain cross Parents	130.40	51.01	16.40	100	80	10
Nested Parents	96.00	46.02	18.90	100	81	16
AP13	-	56.60	-	-	66	-
Alamo	-	43.04	-	-	83	-

Table 3A.6. Quantitative trait loci (QTL) for biomass yield, seed weight, and seed germination identified from the Nested Association Mapping (NAM) population through Composite Interval Mapping (CIM).

Trait	QTL	Chromosome	Position (cM)	LOD	CI[¶]	Nearest SNP	PVE[#]	Additive Effect [††]
Biomass Yield (kg plant^{-1}) [†]	*qBYIa.1*	Ia	12.61	7.6	10.6-14.2	c1a_70541448****	12.5	0.1031
	qBYIa.1	Ia	23.11	3.9	22.9-24.3	c1a_70128273	8.5	-0.0797
	qBYIa.1	Ia	40.71	3.6	38.7-41.8	c1a_71637156	5.5	-0.0445
	qBYIIb.1	IIb	93.9	2.6	86.6-101.6	c2b_61963807****	5.8	0.0334
	qBYIVa.1	IVa	25.3	3.2	21.3-28.2	c4a_1909672****	6.2	0.0383
	qBYIVa.2	IVa	31.2	2.8	28.2-32.8	c4a_4417407****	5.8	0.0339
	qBYVa.1	Va	134.1	3.3	128.3-140.6	c5a_2026388****	6.0	0.0363
	qBYVa.2	Va	155.8	4.9	151.7-159.4	c5a_8191869****	6.7	0.0444
	qBYVa.3	Va	161.6	4.7	159.4-163.2	c5a_8191879****	7.1	0.0485
	qBYVa.4	Va	170.1	4.8	166.7-172.5	c5a_15120636*****	7.0	0.0475
	qBYVa.5	Va	177.8	5.2	174.3-185.7	c5a_3277815****	7.7	0.0535
	qBYVb.1	Vb	20.8	3.0	17.7-24.6	c5b_28806521****	6.5	0.0424
	qBYVIIa.1	VIIa	156.6	2.7	148.8-159.6	c7a_5007502	12.3	-0.0845
	qBYVIIb.1	VIIb	41.0	2.9	39.1-44.8	c7b_345287****	4.6	0.0342
	qBYVIIb.2	VIIb	49.8	4.0	49.8-50.9	c7b_1657299****	5.2	0.0406
	qBYVIIIa.1	VIIIa	60.7	2.7	51.9-64.9	c8a_11913582	6.2	-0.0413
	qBYVIIIa.2	VIIIa	69.1	3.1	64.9-70.8	c8a_11912568	6.3	-0.0431
	qBYVIIIa.3	VIIIa	86.1	4.9	84.3-89.2	c8a_18503891****	5.9	0.0486
	qBYIXa.1	IXa	93.0	4.1	92-93.6	c9a_84435138****	6.0	0.0355
	qBYIXa.2	IXa	105.4	3.1	104.9-106.5	c9a_53017202****	5.6	0.0312
	qBYIXb.1	IXb	132.9	2.7	131.2-139.8	c9b_8351032***	5.6	0.0299
Biomass Yield (kg plant^{-1}) [‡]	*qBYknxIIb.1*	IIb	164.91	3.2	162.4-166.6	c2b_40987348**	3.5	-0.0416
	qBYknxIXa.1	IXa	105.41	4.2	104.6-106.6	c9a_53017202****	3.4	0.0424

Table 3A.6. (continued).

Trait	QTL	Chromosome	Position	LOD	CI[¶]	Nearest SNP	PVE[#]	Additive Effect[††]
Biomass Yield (kg plant-1) [§]	*qBYard.Ia*	Ia	12.3	4.0	2.3-14	c1a_71531924****	10.9	0.0751
	qBYard.IVa	IVa	25.3	2.5	20.8-27.8	c4a_1909672****	9.1	0.0479
	qBYard.IVb	IVb	64.7	2.6	60.4-79.2	c4b_40512089****	10.9	0.0746
	qBYard.Va	Va	46.9	2.6	46.5-48.1	c5a_9378516****	8.6	0.0371
	qBYard.Va	Va	158.7	8.6	155.8-159.4	c5a_16084879****	10.2	0.0884
	qBYard.Vb	Vb	77.1	4.4	76.5-80	c5b_20667870****	8.2	0.0518
	qBYard.Vb	Vb	85.1	2.8	83-86.8	c5b_32327026****	8.0	0.0473
	qBYard.Vb	Vb	93.7	2.6	93.2-97.1	c5b_35721357	8.9	-0.0474
	qBYard.VIb	VIb	48	3.7	46-49.6	c6b_3764436****	9.4	0.0535
	qBYard.VIIa	VIIa	153.6	3.0	146.9-159.6	c7a_5007502*	17.5	-0.1321
	qBYard.VIIb	VIIb	49.8	5.7	49.8-50.9	c7b_1657299****	8.4	0.0563
	qBYard.VIIIa	VIIIa	69.1	6.5	65.6-70.6	c8a_11912568	9.9	-0.0883
	qBYard.VIIIa	VIIIa	85.1	12.3	84.3-87.5	c8a_18503891****	11.6	0.1241
	qBYard.IXb	IXb	76.6	2.4	74.9-78.5	c9b_888160****	8.6	0.0399
Seed Weight (mg 100 seed^{-1})	*qSWVIIIb.1*	VIIIb	10.9	2.7	0-20.3	c8b_8355808**	5.2	-4.5263
	qSWVIIIb.2	VIIIb	67.8	2.8	64.3-74.7	c8b_14530598**	4.6	3.6853
Germination (%)	*qGRMIXa.1*	IXa	176.1	3.5	174.8-180.3	c9a_73835923**	5.2	0.0297

[†] Biomass yield from 2 locations in 2 years.
[‡] Biomass yield from Knoxville in 2 years.
[§] Biomass yield from Ardmore in 2 years.
[¶] 95% confidence interval
[#] Phenotypic variation explained.
[††] Additive effect in kg plant^{-1}.
[*] Significant at P<0.05 from SMA.
[**] Significant at P<0.01 from SMA.
[***] Significant at P<0.001 from SMA.
[****] Significant at P<0.001 from SMA.

Table 3A.7. Sequence similarity with predicted genes from different crops.

QTL	Species	Common Name	Description	Identity	E-value	Accession
qBYIa.1	*Setaria italica*	Foxtail millet	Catalase isozyme 3, predicted	94%	0.00	XM_004952101.2
	Saccharum officinarum	Sugarcane	Catalase (CAT1)	91%	0.00	KF528830.1
	Zea mays	Corn	Catalase (CAT3)	88%	0.00	L05934.1
	Sorghum bicolor	Sorghum	Catalase isozyme 3, predicted	91%	0.00	XM_021460018.1
	Oryza sativa ssp. Indica	Rice	Catalase (CatA)	87%	0.00	EF371902.2
qBYVa.5	*Setaria italica*	Foxtail millet	DEAD-box ATP-dependent RNA helicase 39, predicted	94%	0.00	XM_004968400.4
	Sorghum bicolor	Sorghum	DEAD-box ATP-dependent RNA helicase 39, predicted	87%	3E-152	XM_002455001.2
	Zea mays	Corn	DEAD-box ATP-dependent RNA helicase, putative	86%	6E-140	NM_001136950.1
	Aegilops tauschii	Tausch's goatgrass	DEAD-box ATP-dependent RNA helicase 39, predicted	82%	1E-101	XM_020299084.1
qBYVa.2	*Setaria italica*	Foxtail millet	WRKY transcription factor 12, predicted	78%	1E-106	XM_004968384.2
	Zea mays	Corn	WRKY transcription factor (WRKY45)	94%	7E-65	KJ726908.1
	Oryza sativa ssp. japonica	Rice	WRKY transcription factor 79 (WRKY79)	90%	4E-62	BK005213.1
	Sorghum bicolor	Sorghum	WRKY transcription factor 12, predicted	92%	1E-61	XM_002455008.2
	Hordeum vulgare ssp. vulgare	Barley	WRKY transcription factor 14 (WRKY14)	93%	2E-60	DQ840413.1
SWVIIIb.1	*Setaria italica*	Foxtail millet	Polygalacturonase At1g80170, predicted	89%	5E-16	XM_012844893.2
	Aegilops tauschii	Tausch's goatgrass	Polygalacturonase At1g80170, predicted	85%	5E-11	XM_020345316.1
GRMIXa.1	*Setaria italica*	Foxtail millet	Zinc finger protein 4, predicted	89%	9E-178	XM_022829854.1
	Zea mays	Corn	Zinc finger protein 7, predicted	78%	4E-67	XM_023302467.1
	Sorghum bicolor	Sorghum	Zinc finger protein 4, predicted	82%	2E-64	XM_021463576.1

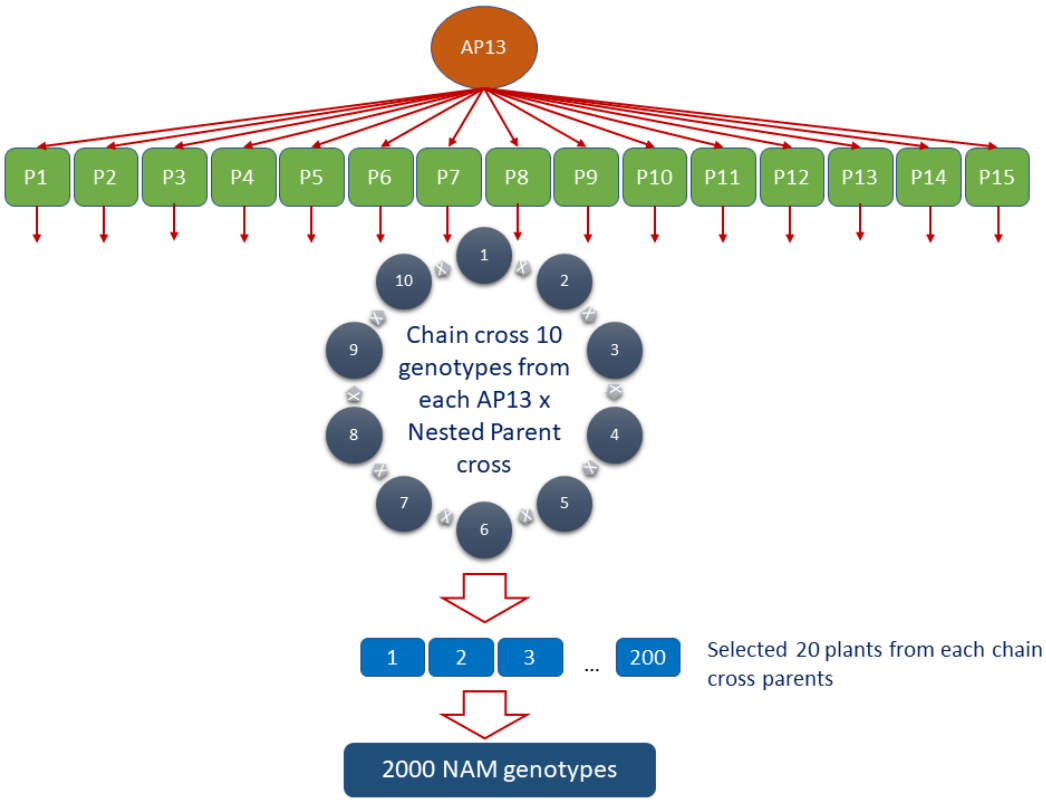


Figure 3A.1. Schematic diagram of Nested Association Mapping (NAM) population material development.

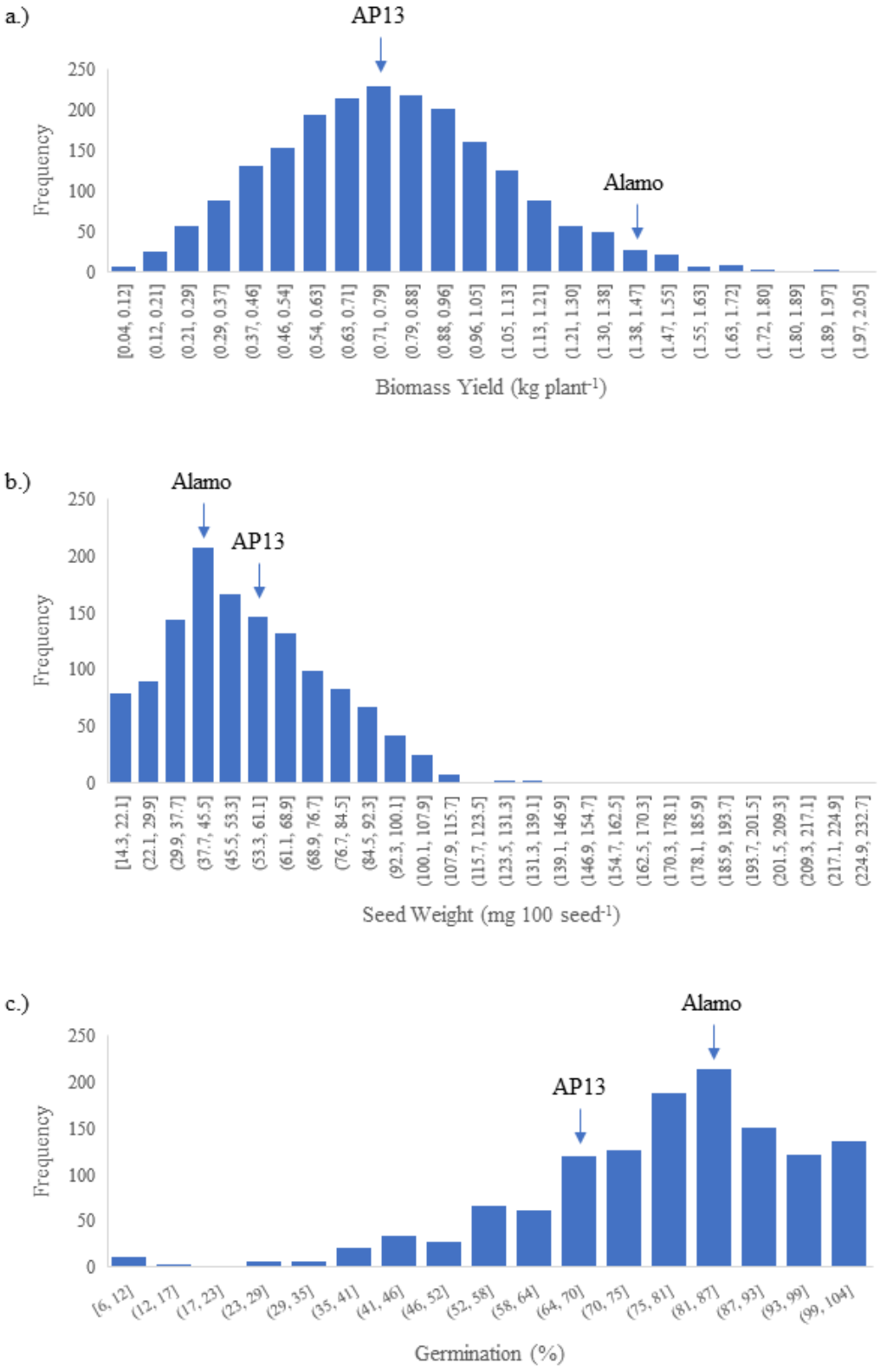


Figure 3A.2. Frequency distribution of Nested Association Mapping Population for (a.) biomass yield across 2 locations (Knoxville and Ardmore) and 2 years (2014-2015) and (b.) seed weight and (c) seed germination across two locations (Knoxville and Ardmore) in 2015.

Figure 3A.3. Genetic map generated using 2684 SNPs and a Nested Association Mapping (NAM) population.

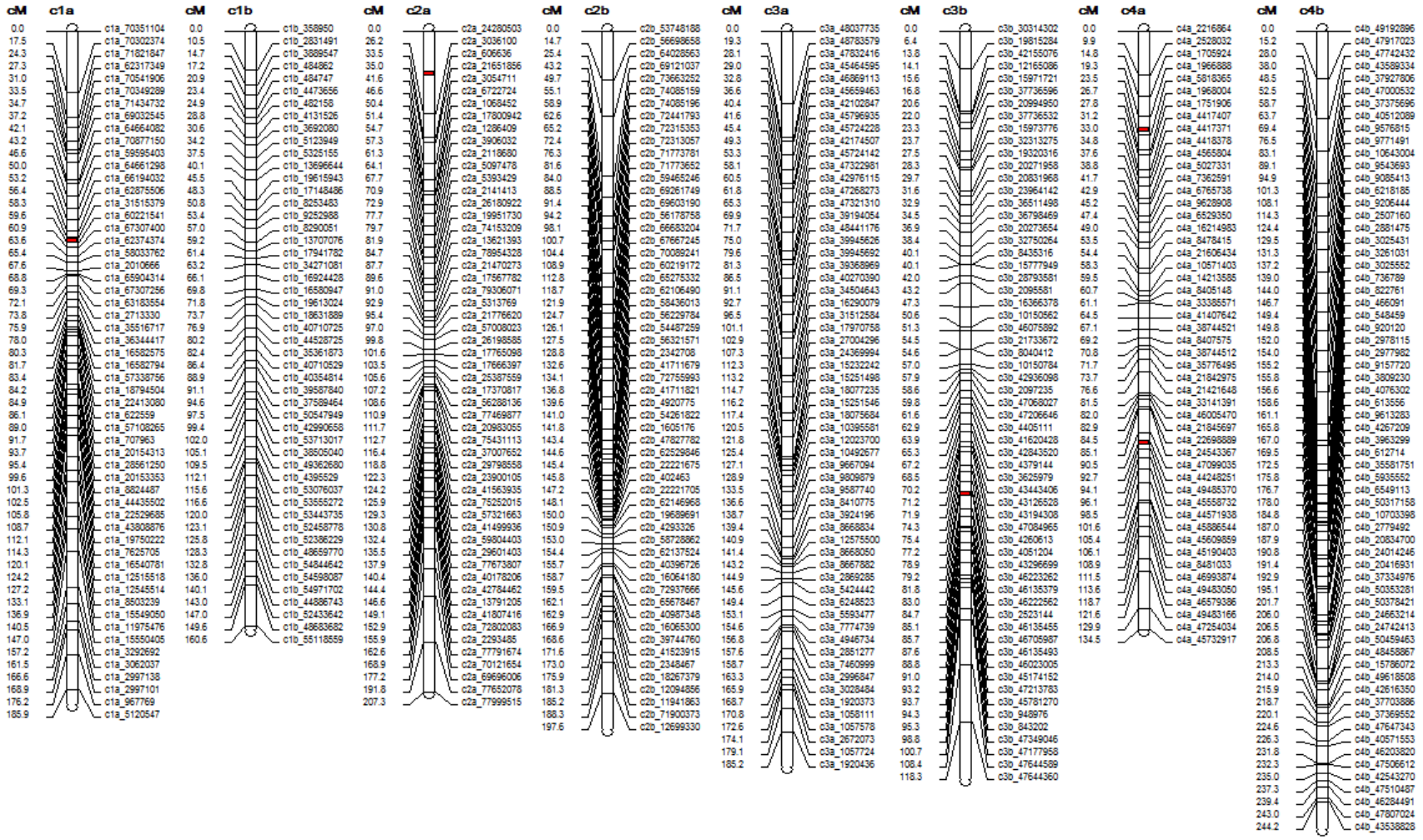


Figure 3A.3. (continued).

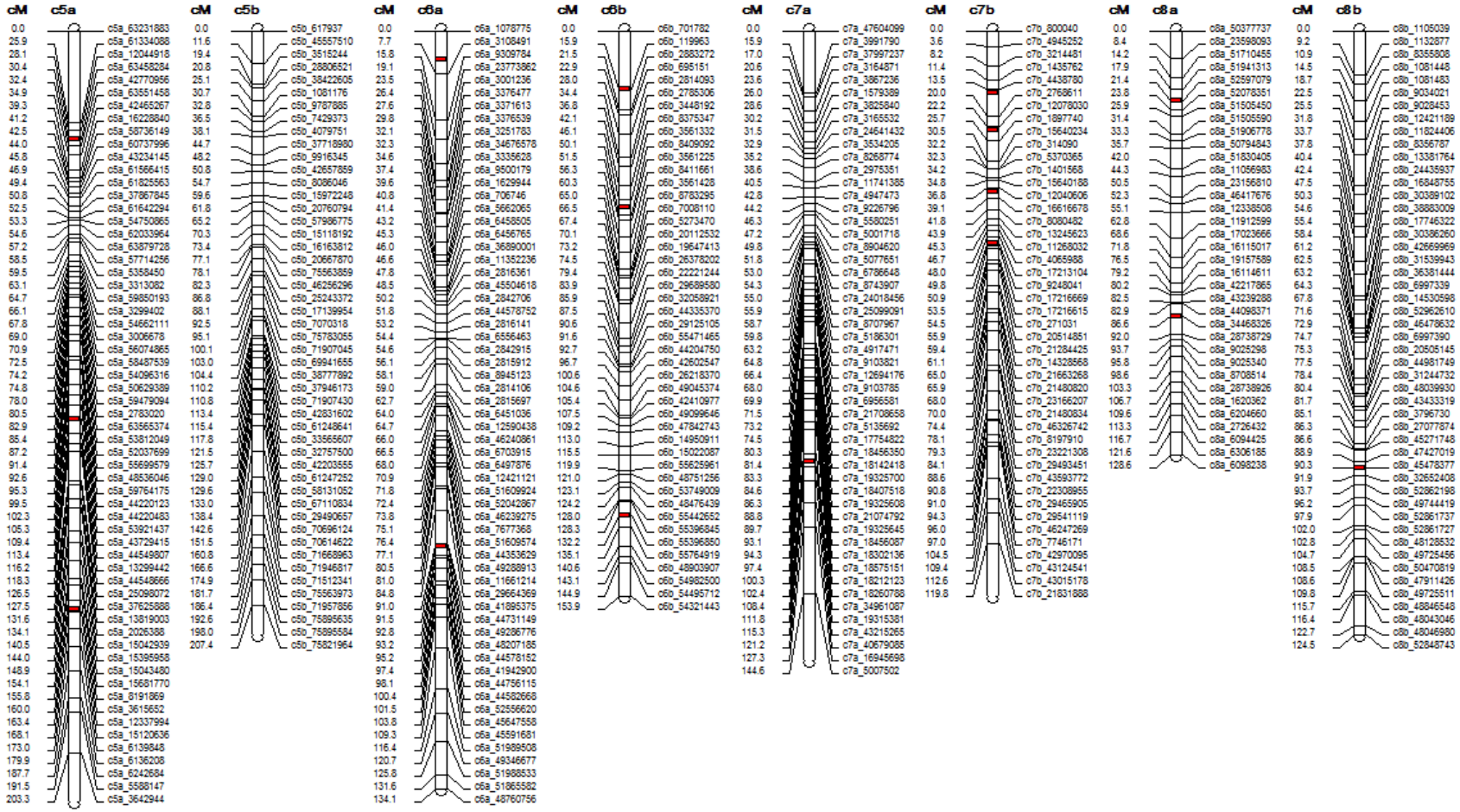


Figure 3A.3. (continued).

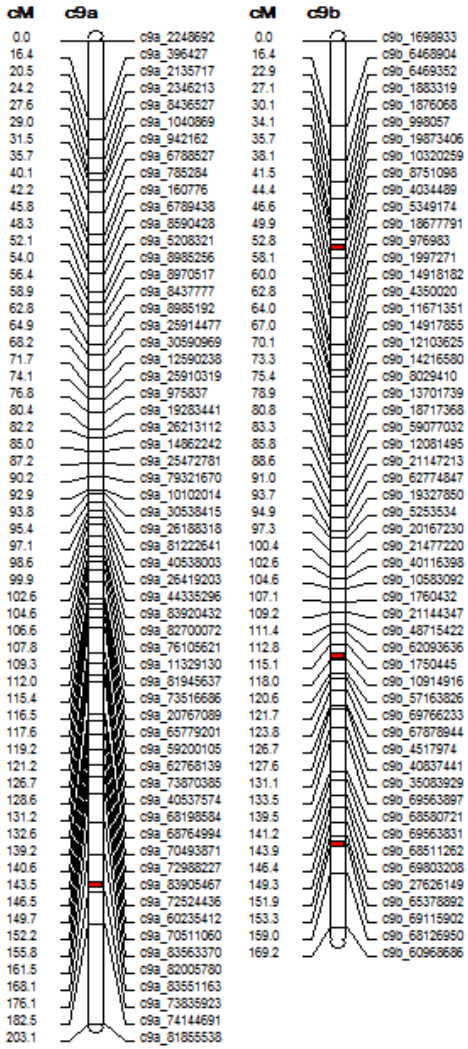


Figure 3A.3. (continued).

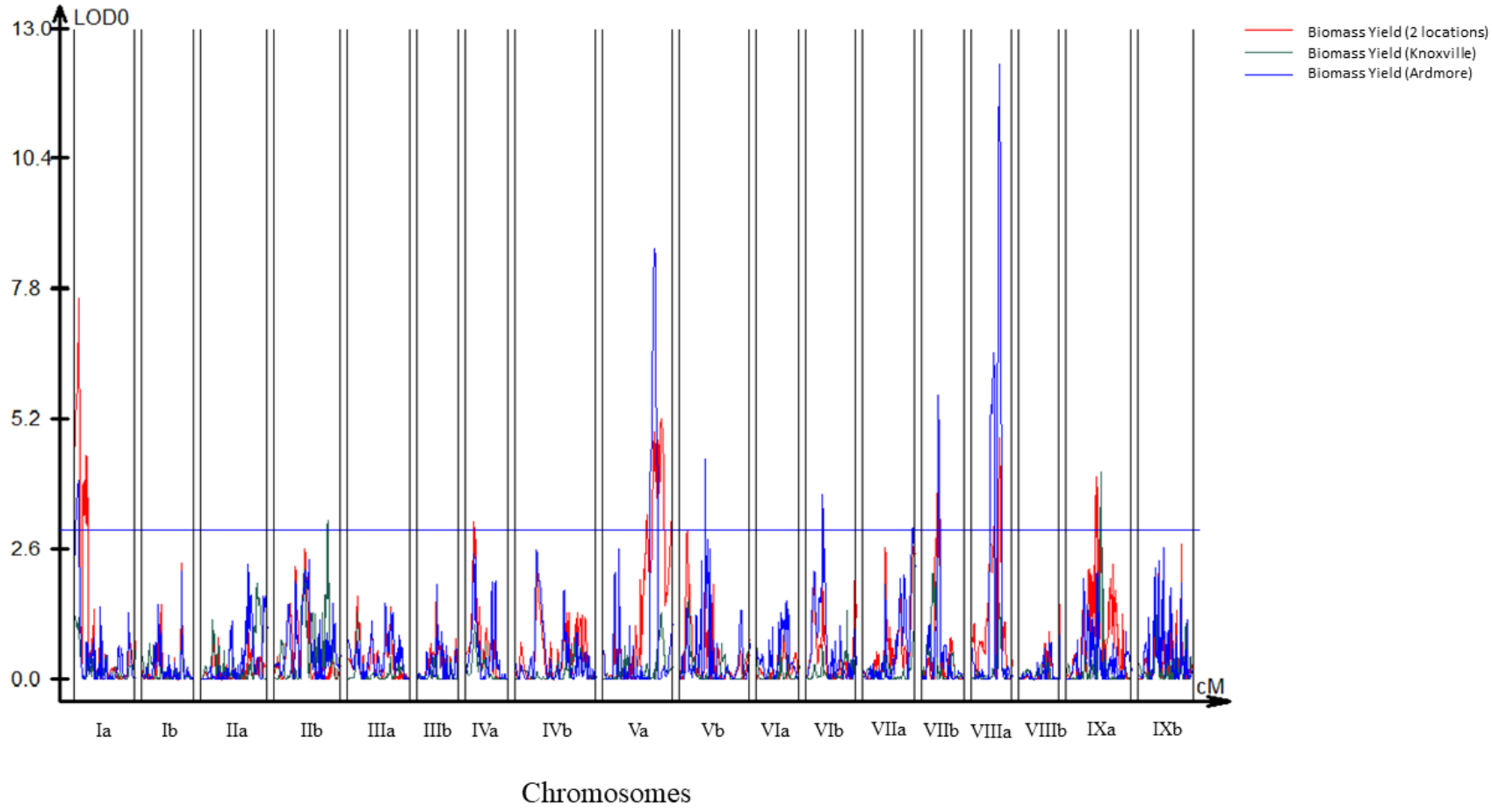


Figure 3A.4. Quantitative trait loci (QTL) detected using Composite Interval Mapping (CIM) from the Nested Association Mapping (NAM) population for biomass yield across two locations, in Knoxville and in Ardmore in two years (2014-2015).

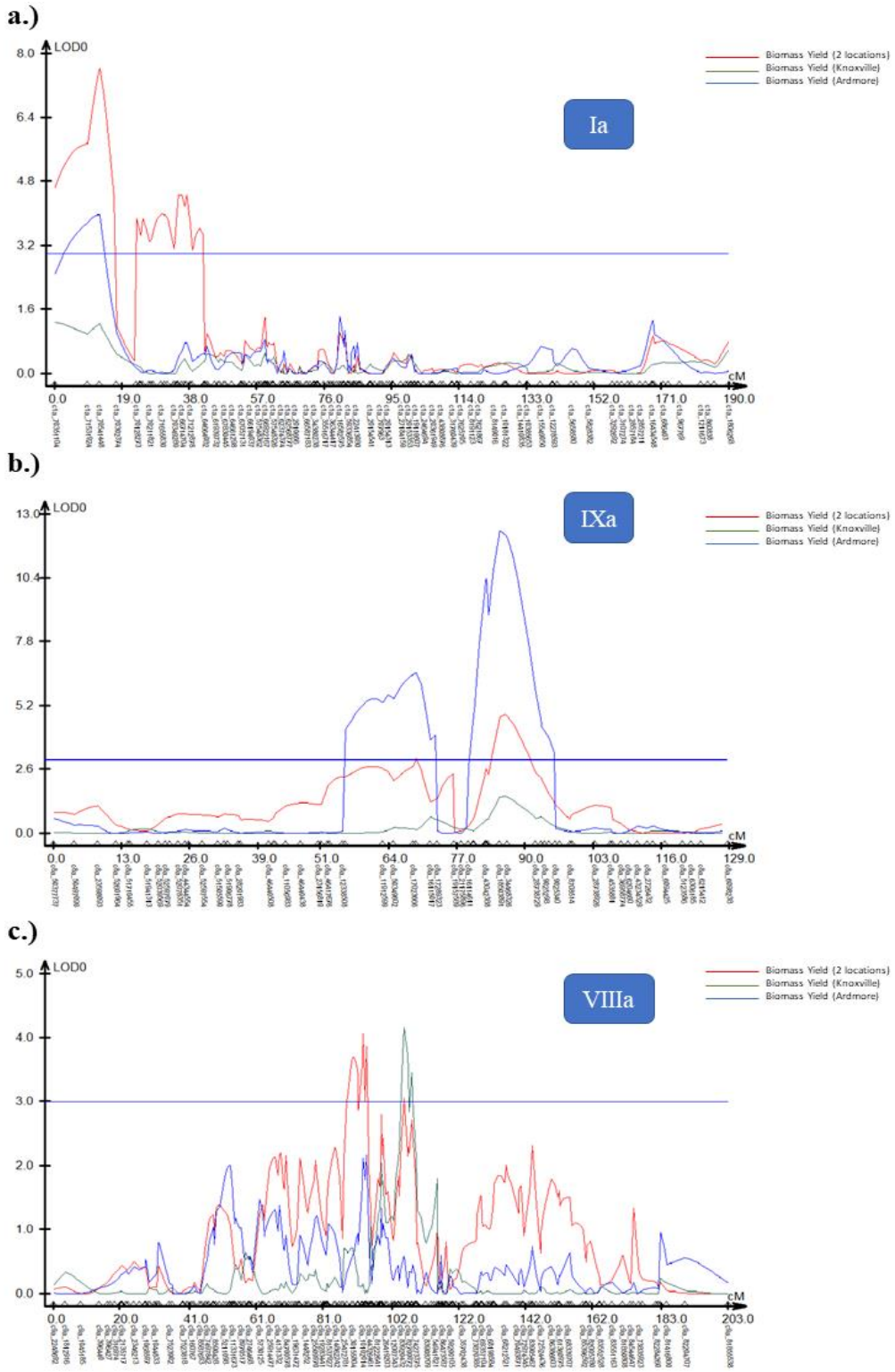


Figure 3A.5. Quantitative trait loci (QTL) detected using Composite Interval Mapping (CIM) from the Nested Association Mapping (NAM) population for biomass yield with the highest LOD score located in (a.) in chromosome Ia across two locations, (b.) chromosome IXa in Knoxville, and (c.) chromosome VIIIa in Ardmore in 2 years (2014-2015).

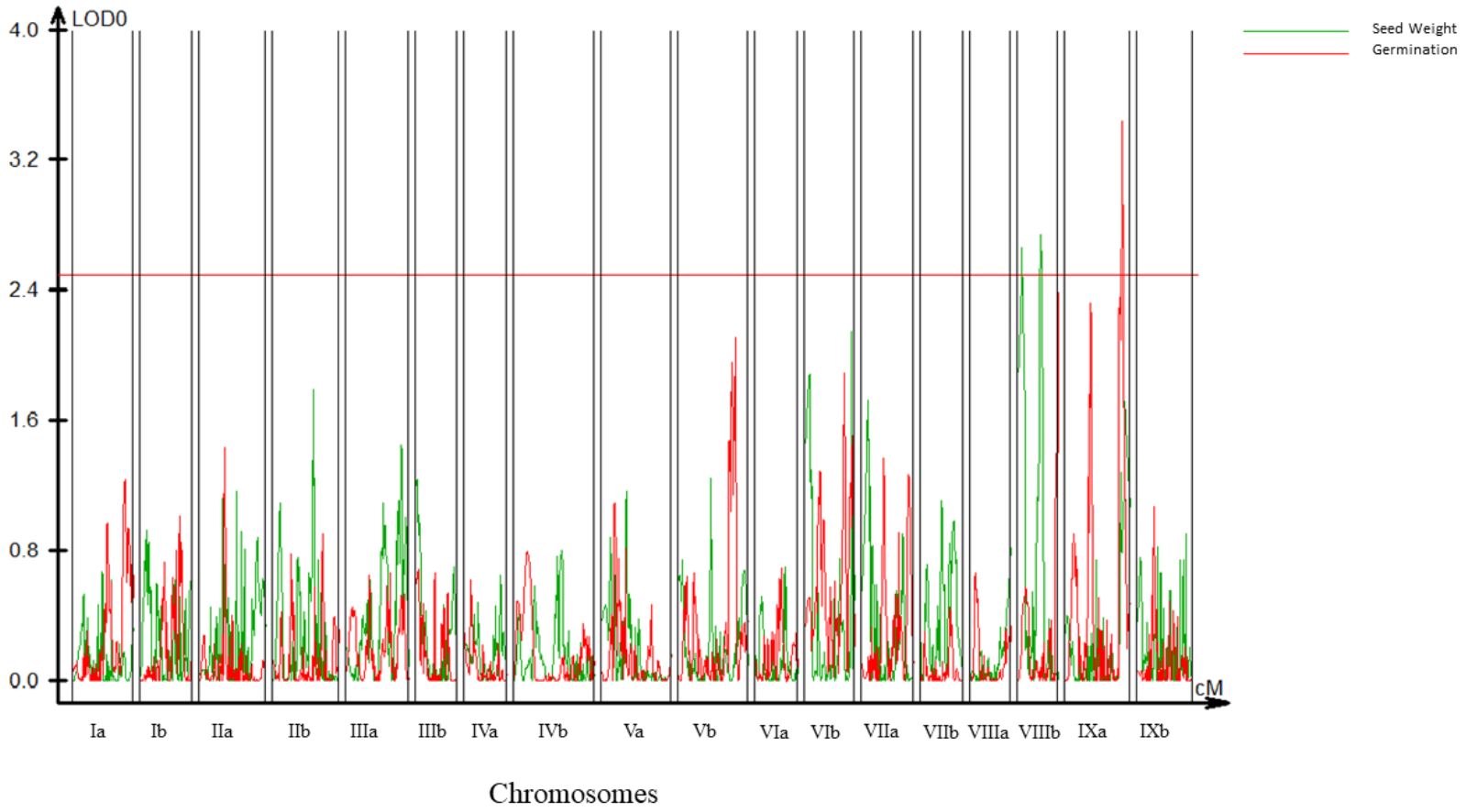
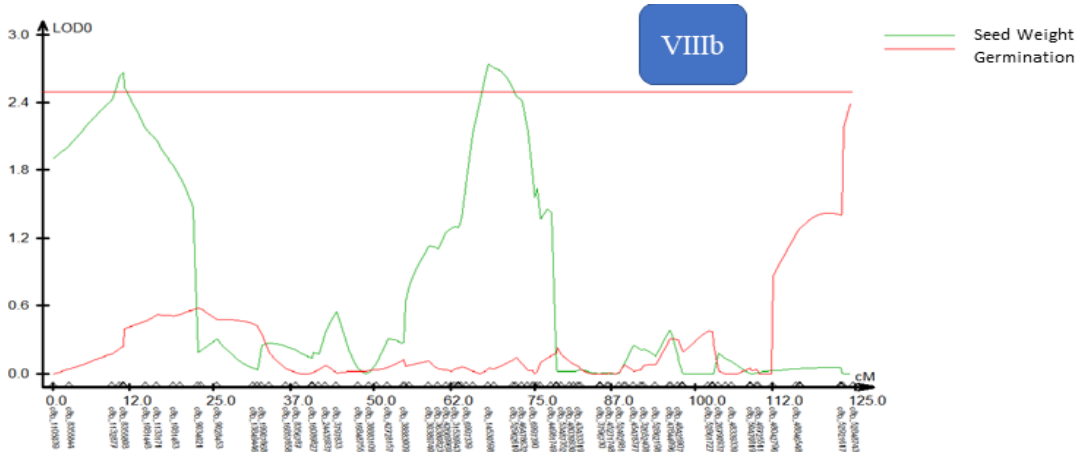


Figure 3A.6. Quantitative trait loci (QTL) detected using Composite Interval Mapping (CIM) from the Nested Association Mapping (NAM) population for seed weight and germination across locations in 2015.

a.)



b.)

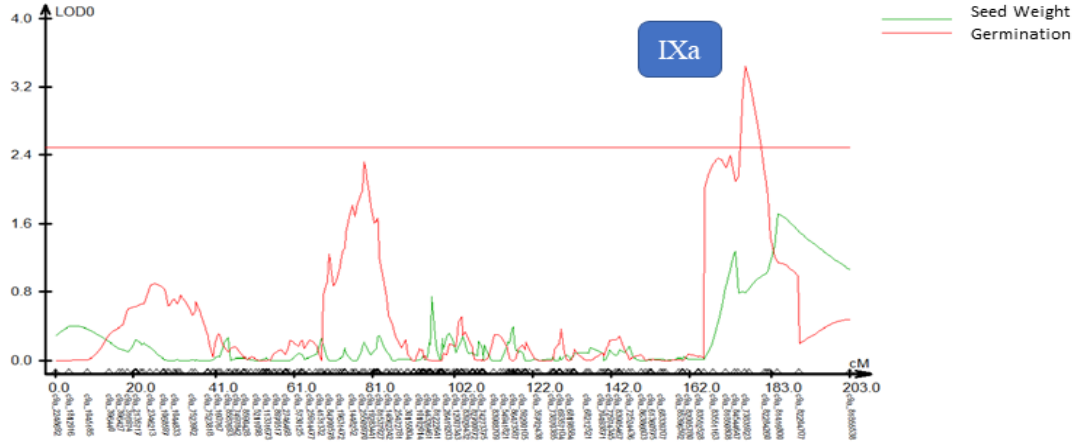


Figure 3A.7. Quantitative trait loci (QTL) detected using Composite Interval Mapping (CIM) from the Nested Association Mapping (NAM) population with the highest LOD score located in (a.) chromosome VIIIb for seed weight (b.) and chromosome IXa for germination in 2015.

APPENDIX 3B

CHAPTER 3 SUPPLEMENTAL TABLES

Table 3B. List of associated SNPs from single marker analysis (SMA) and Interval Mapping (IM).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c1a_70351104	0.00	c1a_70351104	0.00	c1a_71531924	9.31	c1a_70349289	33.49	c1a_20361949	106.42
	c1a_71531924	9.31	c1a_71531924	9.31	c1a_70541448	12.62	c1a_20382293	92.14	c1a_2998534	109.48
	c1a_70541448	12.62	c1a_70541448	12.62	c1a_70302374	17.49	c1a_33538135	92.52	c1a_3292692	157.24
	c1a_70302374	17.49	c1a_70302374	17.49	c2a_3875404	34.28	c1b_1928481	14.58	c1a_7109681	158.63
	c1b_8289985	46.41	c1a_62374374	63.60	c2a_74128163	34.46	c1b_3889547	14.65	c1a_3107274	159.67
	c1b_17148486	48.34	c1a_66921256	64.28	c2a_14964147	34.62	c1b_2832868	17.17	c1a_3062037	161.51
	c1b_593061	48.37	c1a_68872996	64.47	c2a_21651856	34.97	c1b_484862	17.25	c1a_2857184	162.50
	c1b_9182379	48.41	c1a_18794504	84.15	c2a_2180150	37.73	c1b_2691794	17.75	c1a_2857211	164.88
	c1b_8290479	48.51	c1a_59336858	84.36	c2a_74128611	41.43	c1b_21815275	18.22	c1a_2997138	166.57
	c1b_8253483	50.77	c1a_44981645	84.52	c2a_26198585	99.82	c1b_1888492	19.35	c1a_16434548	168.34
	c1b_8769232	56.70	c1a_22413080	84.87	c2a_77465634	100.38	c1b_1796084	22.38	c1a_686403	171.50
	c1b_8290051	57.02	c1a_18380465	85.31	c2a_13619798	100.74	c1b_4473656	23.44	c1a_3107783	172.96
	c1b_9660118	57.53	c1a_218703	85.89	c2a_15082482	101.33	c1b_2400123	23.56	c1b_52115015	121.80
	c1b_586690	57.97	c1a_622559	86.11	c2a_66447784	131.29	c2a_75351313	72.73	c1b_52266443	121.94
	c1b_34108943	60.06	c1a_36344037	86.43	c2a_42430437	135.02	c2a_13634245	79.66	c1b_52386229	125.81
	c1b_13500715	60.09	c1b_3008913	33.58	c2a_70121654	168.91	c2a_17902779	79.69	c1b_51619419	127.41
	c1b_42375049	98.65	c1b_8932392	33.73	c2a_69963031	169.41	c2a_74153209	79.69	c1b_51556254	128.20
	c1b_35545051	99.77	c1b_5123949	34.16	c2a_73634306	174.14	c2a_23548275	114.30	c1b_48659770	128.35
	c1b_51940471	122.02	c1b_4668687	38.60	c2a_69696006	177.23	c2a_75633522	115.14	c1b_54664994	128.60
	c1b_52458778	123.13	c1b_8932472	38.66	c2a_75043372	180.40	c2a_75252742	131.21	c2a_5368126	75.92
	c2a_74128163	34.46	c1b_7208270	38.94	c2a_75047306	183.19	c2a_70007873	162.93	c2a_9317095	76.16
	c2a_695910	51.44	c1b_13696644	40.08	c2a_77729236	188.55	c2a_3036261	162.97	c2a_16101079	77.03
	c2a_35527082	141.39	c1b_19615943	45.46	c2a_77652078	191.80	c2a_69918605	165.26	c2a_19951730	77.69
	c2a_13791205	146.64	c1b_8932870	45.47	c2b_72315963	50.12	c2a_69696006	177.23	c2a_11778221	77.69
	c2a_42791335	147.28	c1b_8289985	46.41	c2b_69262326	51.48	c2a_75043372	180.40	c2a_13634245	79.66
	c2a_75521126	148.67	c1b_1005294	47.17	c2b_74085159	55.12	c2a_77469178	198.04	c2a_17902779	79.69
	c2a_62375434	148.69	c1b_17148486	48.34	c2b_71007666	90.09	c2b_62521788	19.46	c2a_74153209	79.69
	c2a_41807416	149.07	c1b_593061	48.37	c2b_38078333	90.66	c2b_70933850	22.26	c2a_17534841	80.06
	c2a_69898181	149.13	c1b_9182379	48.41	c2b_69603190	91.35	c2b_64028563	25.41	c4a_1966888	19.34
	c2a_1209378	149.93	c1b_8290479	48.51	c2b_69262112	92.09	c2b_74043014	75.15	c4b_43589334	37.97

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c2a_75521174	151.23	c1b_8253483	50.77	c2b_61963807	93.92	c2b_71773781	76.27	c5a_12731790	35.18
	c2a_72802083	152.91	c1b_8284480	51.89	c2b_56178758	94.15	c2b_72328548	77.10	c5a_61313221	37.92
	c2a_69729178	153.69	c1b_720529	52.42	c2b_69120177	96.85	c2b_70108689	78.17	c5a_42465267	39.29
	c2a_77652148	154.64	c1b_16678542	53.09	c2b_73002333	127.41	c2b_70879843	82.15	c5a_58735889	40.28
	c2a_2293485	155.95	c1b_8769232	56.70	c2b_57393782	147.59	c2b_65542636	124.03	c5a_16228840	41.18
	c2b_69814707	34.31	c1b_8290051	57.02	c2b_62146968	148.14	c2b_56321507	124.57	c5b_66642543	116.76
	c2b_73591076	42.99	c1b_9660118	57.53	c2b_5631272	148.72	c2b_56229784	124.70	c5b_70696124	142.64
	c2b_69121037	43.17	c1b_45652352	98.45	c2b_39831588	149.93	c2b_1427358	125.54	c5b_57940602	143.08
	c2b_74043151	45.53	c1b_42375049	98.65	c2b_19689691	149.98	c2b_54374031	126.11	c5b_71658350	147.18
	c2b_65464384	46.75	c1b_35545051	99.77	c2b_41763475	150.10	c2b_54487259	126.13	c5b_70614622	151.48
	c2b_73663252	49.73	c1b_51940471	122.02	c2b_62137907	150.27	c2b_58652018	126.86	c5b_71098491	152.28
	c2b_72315963	50.12	c1b_54972112	136.95	c2b_4293326	150.91	c2b_73002333	127.41	c5b_67110012	156.23
	c2b_69262326	51.48	c1b_53801546	138.74	c2b_5131328	152.02	c2b_56321571	127.46	c5b_71668963	160.75
	c2b_74085159	55.12	c1b_54971702	140.14	c2b_72932108	152.90	c2b_54486925	128.68	c5b_71514101	165.01
	c2b_72441793	62.61	c2a_21470273	87.72	c2b_58728862	153.04	c3a_24369994	107.35	c5b_71946817	166.57
	c2b_73498319	64.49	c2a_17590861	88.37	c2b_4384807	153.61	c3a_16037949	111.64	c5b_69532892	170.23
	c2b_73863339	65.10	c2a_13619867	88.76	c2b_33152667	153.85	c3a_15232242	112.31	c5b_71891790	174.90
	c2b_72315353	65.18	c2a_423753	89.33	c2b_62137524	154.37	c3a_22872672	112.39	c5b_71512341	174.93
	c2b_73980055	67.68	c2a_17567782	89.60	c2b_8303634	162.61	c3a_15251498	113.16	c5b_74319947	176.25
	c2b_70995140	70.98	c2a_11690545	89.62	c2b_40987348	162.95	c3a_25908783	114.22	c5b_71892178	178.86
	c2b_72313057	72.35	c2a_11690508	90.22	c2b_8315999	165.94	c3a_18077235	114.74	c5b_75563973	181.68
	c2b_72328530	72.90	c2a_16153946	90.84	c2b_39752428	166.63	c3a_10397325	116.08	c5b_71817649	182.45
	c2b_71773652	81.63	c2a_79306071	90.95	c2b_16065300	166.86	c3a_15251546	116.21	c6b_3211697	41.10
	c2b_70156297	82.09	c2a_13619420	91.56	c2b_65463834	166.88	c3a_17970093	116.96	c6b_55625961	119.86
	c2b_70879843	82.15	c2a_70104482	91.78	c2b_39744760	168.61	c3a_18075684	117.40	c6b_49788328	120.61
	c2b_59465246	84.03	c2a_79197347	92.12	c2b_18217668	168.88	c3a_16123079	120.24	c6b_48751256	121.01
	c2b_70168759	85.21	c2a_5313769	92.90	c3a_48763659	28.19	c3a_10395581	120.50	c6b_49289292	121.34
	c2b_69505322	86.99	c2a_15612482	93.60	c3a_45464595	29.03	c3a_16037828	121.59	c6b_53749009	123.08
	c2b_69261749	88.51	c2a_15302987	94.74	c3a_45579817	30.59	c3a_12023700	121.78	c6b_56090304	123.22
	c2b_71007666	90.09	c2a_17567733	95.09	c3a_46869113	32.82	c3a_8273690	123.36	c6b_54495712	144.89

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c2b_38078333	90.66	c2a_21776620	95.38	c3a_48783410	34.88	c3a_10492677	125.42	c6b_48903756	146.54
	c2b_69603190	91.35	c2a_25731938	95.83	c3b_1300380	57.73	c3a_10518538	125.68	c7a_5077821	66.20
	c2b_69262112	92.09	c2a_17410755	96.05	c4a_5818365	23.48	c3a_9667094	127.12	c7a_12694176	66.39
	c2b_61963807	93.92	c2a_15303141	96.06	c4a_1909672	25.26	c3a_24408911	127.19	c7a_23639836	66.81
	c2b_56178758	94.15	c2a_57008023	97.00	c4a_1968004	26.66	c3a_13361050	156.10	c7a_5135730	67.79
	c2b_69120177	96.85	c2a_15171857	97.47	c4a_7148865	27.46	c3a_4946734	156.76	c7a_9103785	68.04
	c2b_63727134	97.60	c2a_40107938	97.85	c4a_1751906	27.81	c3a_2998896	157.03	c7a_8206670	68.30
	c2b_66683204	98.11	c2a_69705058	98.81	c4a_2551307	28.18	c3a_2996847	163.35	c7a_23337859	69.42
	c2b_17676600	99.67	c2a_26198585	99.82	c4a_4417407	31.17	c3a_1730535	163.87	c7a_8936543	112.65
	c2b_63917886	99.85	c2a_75633522	115.14	c4a_7149110	32.16	c3a_2606484	167.78	c7a_16773064	112.70
	c2b_67667245	100.67	c2a_11190087	116.87	c4a_6765738	42.88	c3a_1920373	168.73	c7a_10334788	140.38
	c2b_62622625	103.45	c2a_46622314	124.77	c4a_7235483	44.24	c3a_13672715	169.31	c7a_5007502	144.61
	c2b_63953589	104.36	c2a_24174472	125.30	c4a_9628908	45.21	c3a_1058111	170.84	c8b_47427019	88.90
	c2b_70089241	104.36	c2a_11813094	125.80	c4a_21842058	60.94	c3a_1339280	170.99	c8b_48412220	89.15
	c2b_59964453	104.39	c2a_75252015	125.93	c4b_18236576	186.25	c3a_1057578	172.57	c8b_51004735	91.49
	c2b_65275379	108.07	c2a_29165982	128.32	c5a_15120636	168.10	c3a_1920436	185.21	c8b_32652408	91.86
	c2b_60219172	108.89	c2a_75252742	131.21	c5a_3505220	171.05	c3b_30314302	0.00	c8b_52787795	91.91
	c2b_20612209	179.98	c2a_66447784	131.29	c5a_3662227	171.48	c3b_41637671	3.06	c8b_52862198	93.72
	c2b_21851537	180.06	c2a_4782916	131.31	c5a_6139848	173.02	c3b_47644360	118.30	c8b_47844096	95.96
	c2b_12094856	181.32	c2a_59804403	132.44	c5a_3277815	177.82	c4a_49483050	113.56	c8b_49744419	96.15
	c2b_11803249	182.23	c2a_42430437	135.02	c5a_3598780	178.78	c4b_47917023	15.24	c8b_48425907	97.29
	c2b_11996818	201.16	c2a_75188418	135.16	c5a_6136208	179.86	c4b_40512089	63.66	c8b_52861737	97.89
	c2b_5547788	208.07	c2a_66332282	135.35	c5a_10674591	180.19	c5a_60264051	73.55	c8b_2933221	98.10
	c3a_48783579	19.29	c2a_29601403	135.46	c5a_3258606	186.30	c5a_54096316	74.17	c8b_52861727	102.02
	c3a_48006186	19.65	c2a_618575	136.87	c5a_6242684	187.65	c5a_51588166	74.30	c8b_48425987	102.59
	c3a_47832416	28.05	c2a_69966563	137.44	c5b_12437247	19.85	c5a_59477569	74.36	c8b_48128532	102.82
	c3a_48763659	28.19	c2a_41711146	137.69	c5b_981486	20.04	c5a_50629389	74.76	c8b_48846548	115.70
	c3a_45464595	29.03	c2a_77673807	137.90	c5b_4731274	23.71	c5b_42687892	99.77	c8b_48239173	116.15
	c3a_45579817	30.59	c2a_47506906	139.25	c5b_1507378	24.34	c5b_71907045	100.11	c8b_48043046	116.43
	c3a_46869113	32.82	c2a_25566660	139.26	c5b_38422605	25.14	c6a_41895375	91.00	c8b_52921617	122.60

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c3a_48783410	34.88	c2a_34190682	140.05	c5b_658965	25.41	c6a_44927383	91.17	c8b_48046980	122.69
	c3a_45659463	36.57	c2a_40178206	140.42	c5b_4276920	27.92	c6a_7458078	94.25	c8b_48239385	123.01
	c3a_39785719	70.66	c2a_35527082	141.39	c5b_1081176	30.69	c6a_44578152	95.20	c8b_52848743	124.46
	c3a_48441176	71.75	c2a_79416402	141.43	c6b_3764436	48.04	c6a_44043545	95.26	c9a_2346213	24.22
	c3a_36372270	72.92	c2a_37582554	143.06	c6b_8409092	50.06	c6b_701782	0.00	c9a_1026754	25.99
	c3a_39945626	74.97	c2a_42784462	144.43	c6b_55552809	125.56	c6b_701219	11.89	c9a_1068597	27.53
	c3a_39846719	77.05	c2a_69695108	145.47	c7b_15640234	30.54	c6b_119963	15.93	c9a_8436527	27.55
	c3a_39945692	79.59	c2a_604879	145.61	c7b_12052754	31.14	c6b_687575	16.89	c9a_853405	27.88
	c3a_34504643	91.08	c2a_77993010	145.62	c7b_314090	32.17	c6b_2883272	21.49	c9a_1044833	30.32
	c3a_41259198	91.38	c2a_13791205	146.64	c7b_17408929	32.25	c6b_2971897	22.28	c9a_1916065	31.95
	c3a_16290079	92.68	c2a_42791335	147.28	c7b_5370365	32.27	c6b_695151	22.88	c9a_9399894	35.76
	c3a_15251498	113.16	c2a_75521126	148.67	c7b_1980230	33.62	c6b_44335370	87.47	c9a_25910319	74.07
	c3a_25908783	114.22	c2a_62375434	148.69	c7b_1401568	34.24	c6b_55630164	142.32	c9a_1448252	75.75
	c3a_18077235	114.74	c2a_41807416	149.07	c7b_2094164	34.72	c6b_54982500	143.10	c9a_84541172	76.41
	c3a_10397325	116.08	c2a_69898181	149.13	c7b_15640188	34.79	c6b_55679731	143.28	c9a_21335113	76.42
	c3a_15251546	116.21	c2a_1209378	149.93	c7b_14115861	36.70	c6b_54495712	144.89	c9a_975837	76.80
	c3a_17970093	116.96	c2a_75521174	151.23	c7b_12040606	36.78	c7a_3829490	11.93	c9a_25509970	78.45
	c3a_18075684	117.40	c2a_72802083	152.91	c7b_8079929	39.00	c7a_3991790	15.94	c9a_84298315	78.74
	c3a_16037828	121.59	c2a_42791655	153.35	c7b_16616678	39.10	c7a_6293365	16.04	c9a_10102127	79.17
	c3a_12023700	121.78	c2a_69729178	153.69	c7b_345287	40.99	c7a_3403251	16.07	c9a_19283441	80.41
	c3a_10518538	125.68	c2a_77652148	154.64	c7b_8080482	41.78	c7a_37997237	16.96	c9a_81286100	81.41
	c3a_9667094	127.12	c2a_2293485	155.95	c7b_17250208	43.39	c7a_3829506	18.59	c9a_79992653	81.46
	c3a_24408911	127.19	c2a_69588311	156.84	c7b_13245623	43.88	c7a_35500555	19.12	c9a_12474599	81.99
	c3a_9667145	131.15	c2a_13401279	159.72	c7b_4032798	44.97	c7a_3164871	20.64	c9a_26213112	82.20
	c3a_9587740	133.49	c2a_69589855	160.16	c7b_11268032	45.30	c7a_4406871	23.51	c9a_19175466	82.25
	c3a_9488374	134.66	c2a_77791674	162.56	c7b_987527	46.79	c7a_3867236	23.59	c9a_85396592	159.26
	c3a_12526678	138.55	c2a_70007873	162.93	c7b_17213104	47.95	c7a_6086928	23.66	c9a_83634442	160.24
	c3a_24392182	139.12	c2a_3036261	162.97	c7b_18646766	48.72	c7a_5831427	24.44	c9a_80102840	160.36
	c3a_8668834	139.36	c2a_69918605	165.26	c7b_9248041	49.81	c7a_12389889	28.53	c9a_82005780	161.46
	c3b_30360730	39.80	c2a_69696006	177.23	c7b_17216669	50.92	c7a_3825840	28.59	c9a_83552528	164.11

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c3b_8435316	40.06	c2a_77652078	191.80	c7b_48751067	94.68	c7a_4947473	42.83	c9a_86217831	164.42
	c3b_42152868	40.09	c2a_77470461	194.66	c7b_42751101	96.37	c7a_5357467	43.13	c9a_74148909	165.62
	c3b_15777949	40.10	c2a_77469178	198.04	c7b_7746171	97.03	c7a_8958662	43.16	c9a_83551163	168.14
	c3b_35204489	41.68	c2a_76843098	201.69	c8a_17289323	73.20	c7a_3154729	45.42	c9a_81850808	171.19
	c3b_28793581	42.05	c2a_77999515	207.33	c8a_43239288	82.52	c7a_5580251	46.33	c9a_84544647	173.52
	c3b_43443567	56.72	c2b_53748188	0.00	c8a_16829097	82.62	c7a_2258058	46.71	c9a_26327952	174.45
	c3b_10150784	57.01	c2b_61878420	6.18	c8a_44098371	82.88	c7a_8743907	54.32	c9a_73835923	176.14
	c3b_1300380	57.73	c2b_70934935	8.47	c8a_18503891	85.08	c7a_6786680	54.57	c9a_82209298	177.11
	c3b_42936098	57.86	c2b_56698658	14.74	c8a_34468326	86.63	c7a_24332117	54.69	c9a_82284260	181.20
	c3b_17624128	58.03	c2b_64028563	25.41	c8a_14434016	86.64	c7b_2768611	19.99	c9a_81718950	181.89
	c3b_2097235	58.57	c2b_69814707	34.31	c8a_28738729	91.98	c7b_21663268	64.96	c9a_74144691	182.50
	c5a_61825563	49.44	c3a_25908783	114.22						
	c5a_61751442	50.03	c3a_18077235	114.74						
	c5a_61485247	50.18	c3a_10397325	116.08						
	c5a_37867845	50.83	c3a_15251546	116.21						
	c5a_62103865	51.04	c3a_17970093	116.96						
	c5a_51404312	86.71	c3a_18075684	117.40						
	c5a_52037699	87.20	c3a_16123079	120.24						
	c5a_51601576	99.31	c3a_10395581	120.50						
	c5a_44220123	99.53	c3a_16037828	121.59						
	c5a_2396322	99.95	c3a_12023700	121.78						
	c5a_54679529	102.11	c3a_8273690	123.36						
	c5a_53921437	105.30	c3a_10492677	125.42						
	c5a_44216434	106.64	c3a_10518538	125.68						
	c5a_45936852	107.95	c3a_9667094	127.12						
	c5a_43729415	109.40	c3a_24408911	127.19						
	c5a_43680378	111.02	c3a_9667145	131.15						
	c5a_43823742	111.98	c3a_9587740	133.49						
	c5a_1948795	114.32	c3a_9488374	134.66						
	c5a_26098597	114.80	c3a_8410775	136.60						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c5a_13299442	116.15	c3a_12526678	138.55						
	c5a_53921374	116.39	c3a_3924196	138.66						
	c5a_28964748	117.30	c3a_24392182	139.12						
	c5a_44548666	118.30	c3a_8668834	139.36						
	c5a_13861701	120.37	c3a_8095284	140.20						
	c5a_43175327	121.68	c3a_12575500	140.91						
	c5a_25098072	126.50	c3a_8397411	141.01						
	c5a_13857923	127.04	c3a_8668050	141.43						
	c5a_3561939	127.27	c3a_10715682	141.44						
	c5a_37625888	127.50	c3a_8667882	143.15						
	c5a_39246673	129.25	c3a_15309933	143.22						
	c5a_37478203	129.94	c3a_8668986	145.16						
	c5a_13819003	131.59	c3a_6431496	146.24						
	c5a_43680436	131.83	c3a_6248523	149.38						
	c5a_26611568	132.90	c3a_5429283	150.54						
	c5a_2026388	134.05	c3a_5593477	153.11						
	c5a_29424163	137.12	c3a_4963887	153.83						
	c5a_3352157	137.57	c3a_7774739	154.62						
	c5a_15042939	140.54	c3a_2998896	157.03						
	c5a_9165315	140.66	c3a_2851277	157.55						
	c5a_2601216	143.01	c3a_3028862	157.69						
	c5a_15395958	144.03	c3a_7460999	158.70						
	c5a_38901927	147.70	c3a_3103480	159.34						
	c5a_12328356	148.18	c3a_2996847	163.35						
	c5a_15043480	148.91	c3a_1730535	163.87						
	c5a_2016908	152.45	c3a_3028484	165.93						
	c5a_10561141	152.67	c3a_2606484	167.78						
	c5a_15681770	154.12	c3b_36511720	28.01						
	c5a_749728	154.67	c3b_20271958	28.32						
	c5a_6139446	155.76	c3b_32749742	29.34						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c5a_8191869	155.84	c3b_20831968	29.68						
	c5a_16084879	157.65	c3b_30360730	39.80						
	c5a_12437493	159.40	c3b_8435316	40.06						
	c5a_3615652	160.04	c3b_42152868	40.09						
	c5a_8191879	161.56	c3b_15777949	40.10						
	c5a_12159515	163.32	c3b_35204489	41.68						
	c5a_12337994	163.40	c3b_28793581	42.05						
	c5a_6243500	166.40	c3b_46071076	42.93						
	c5a_9150841	167.56	c3b_42779362	45.60						
	c5a_15120636	168.10	c3b_43443567	56.72						
	c5a_3505220	171.05	c3b_10150784	57.01						
	c5a_3662227	171.48	c3b_1300380	57.73						
	c5a_6139848	173.02	c3b_42936098	57.86						
	c5a_3277815	177.82	c3b_17624128	58.03						
	c5a_3598780	178.78	c3b_2097235	58.57						
	c5a_6136208	179.86	c3b_44443671	58.71						
	c5a_10674591	180.19	c3b_47068027	59.81						
	c5a_3258606	186.30	c3b_8042234	60.44						
	c5a_6242684	187.65	c3b_47206646	61.56						
	c5a_35774567	187.79	c3b_8919265	61.83						
	c5a_3599106	191.30	c3b_4405111	62.90						
	c5a_5588147	191.50	c3b_10151081	63.06						
	c5a_5354752	193.79	c3b_41620428	63.92						
	c5a_3299888	198.33	c3b_1308363	63.93						
	c5a_3642944	203.32	c3b_42843520	65.30						
	c5a_2683971	216.34	c3b_4606860	65.73						
	c5a_3234109	216.72	c3b_4379144	67.20						
	c5b_659419	14.27	c3b_8353480	68.25						
	c5b_3515244	19.35	c3b_3625979	68.51						
	c5b_12437247	19.85	c3b_901777	69.36						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c5b_981486	20.04	c3b_43443406	70.25						
	c5b_28806521	20.76	c3b_43194308	71.92						
	c5b_4731274	23.71	c3b_4376235	73.22						
	c5b_1507378	24.34	c3b_47084965	74.28						
	c5b_38422605	25.14	c3b_45113739	74.66						
	c5b_658965	25.41	c3b_4260613	75.44						
	c5b_4276920	27.92	c3b_940853	76.70						
	c5b_1081176	30.69	c3b_1892875	82.22						
	c5b_659708	30.92	c3b_46222562	83.03						
	c5b_4005688	31.36	c3b_1153978	83.56						
	c5b_9787885	32.79	c3b_46135493	87.61						
	c5b_49147428	33.73	c3b_766914	87.71						
	c5b_3145501	35.38	c3b_46023005	88.83						
	c5b_7429373	36.50	c3b_47644360	118.30						
	c5b_64144720	77.07	c4a_2216864	0.00						
	c5b_20667870	77.10	c4a_2497387	7.77						
	c5b_20625690	77.28	c4a_2528032	9.91						
	c5b_14974595	77.60	c4a_1639920	14.20						
	c5b_75563859	78.14	c4a_1705924	14.82						
	c5b_32327026	85.14	c4a_2137519	16.55						
	c5b_36164086	100.69	c4a_1966888	19.34						
	c5b_28277603	101.88	c4a_1956666	22.34						
	c5b_69941655	102.96	c4a_5818365	23.48						
	c5b_71906761	103.29	c4a_1909672	25.26						
	c5b_71957856	186.37	c4a_1968004	26.66						
	c5b_71967588	188.89	c4a_7148865	27.46						
	c5b_75687981	190.18	c4a_1751906	27.81						
	c6a_2843141	46.85	c4a_2551307	28.18						
	c6a_31941374	80.50	c4a_4417407	31.17						
	c6a_11661214	80.98	c4a_7149110	32.16						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c6a_49288868	84.17	c4a_4417371	32.99						
	c6a_29664369	84.76	c4a_5818443	33.09						
	c6a_45731581	87.89	c4a_4418378	34.78						
	c6a_41895375	91.00	c4a_4418495	35.55						
	c6a_41942900	97.38	c4a_4565804	37.61						
	c6a_44756111	97.83	c4a_5002619	38.43						
	c6a_44756115	98.11	c4a_5027331	38.84						
	c6a_49589875	98.93	c4a_5816602	41.43						
	c6b_119963	15.93	c4a_7362591	41.66						
	c6b_687575	16.89	c4a_9863156	41.90						
	c6b_2883272	21.49	c4a_41407642	64.52						
	c6b_2971897	22.28	c4a_21421648	76.56						
	c6b_695151	22.88	c4a_27863786	79.56						
	c6b_700858	25.99	c4a_33141391	81.53						
	c6b_2814093	28.02	c4a_18121450	81.66						
	c6b_2785306	34.43	c4a_46005470	81.97						
	c6b_3211574	34.46	c4a_22191406	82.17						
	c6b_3448192	36.82	c4a_21845697	82.92						
	c6b_3211697	41.10	c4a_21819080	83.61						
	c6b_8375347	42.10	c4a_22698889	84.45						
	c6b_27053215	45.77	c4a_45519577	84.93						
	c6b_3561332	46.08	c4a_24543367	85.12						
	c6b_3764436	48.04	c4a_32988504	90.06						
	c6b_8409092	50.06	c4a_47099035	90.54						
	c6b_17870443	50.60	c4a_29351297	91.14						
	c6b_3561225	51.50	c4a_44248251	92.69						
	c6b_8783275	54.07	c4a_42296977	93.79						
	c6b_8411661	56.32	c4a_22529155	98.48						
	c6b_26218587	57.67	c4a_44571938	98.54						
	c6b_3561428	60.34	c4b_47917023	15.24						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c6b_7007162	60.76	c4b_37375696	58.73						
	c6b_45784842	92.58	c4b_37334382	61.55						
	c6b_44204750	92.70	c4b_40512089	63.66						
	c6b_32074943	95.99	c4b_42612228	66.48						
	c6b_42602547	96.69	c4b_9576815	69.39						
	c6b_44204435	100.42	c4b_36819117	71.51						
	c6b_26218370	100.63	c4b_9771491	76.48						
	c6b_47698023	103.70	c4b_24023487	80.23						
	c6b_54495712	144.89	c4b_10643004	83.10						
	c6b_48903756	146.54	c4b_9705252	85.89						
	c6b_54321443	153.88	c4b_9543693	89.11						
	c7a_3011123	33.66	c4b_822761	143.98						
	c7a_8268774	35.22	c4b_3153015	146.00						
	c7a_3893719	36.37	c4b_466091	146.70						
	c7a_2975351	38.63	c4b_3509870	147.86						
	c7a_11741385	40.49	c4b_548459	149.36						
	c7a_24976636	40.79	c4b_3509881	149.64						
	c7a_4947451	41.33	c4b_920120	149.83						
	c7a_4947473	42.83	c4b_548525	151.61						
	c7a_5357467	43.13	c4b_2978115	151.98						
	c7a_8958662	43.16	c4b_422896	153.32						
	c7a_9226796	44.20	c4b_2977982	153.98						
	c7a_4372034	44.36	c4b_5938112	154.70						
	c7a_5580251	46.33	c4b_9157720	155.15						
	c7a_9103821	64.82	c4b_3192224	155.26						
	c7a_22264020	65.38	c4b_3809230	155.83						
	c7a_5077821	66.20	c4b_548291	155.87						
	c7a_12694176	66.39	c4b_4076302	156.56						
	c7a_23639836	66.81	c4b_3116840	158.09						
	c7a_5135730	67.79	c4b_613556	158.64						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c7a_9103785	68.04	c4b_3809389	159.49						
	c7a_8206670	68.30	c4b_9613283	161.06						
	c7a_23337859	69.42	c4b_6233572	164.83						
	c7a_6956581	69.93	c4b_4267209	165.77						
	c7a_8422837	70.66	c4b_23411331	166.63						
	c7a_10814898	71.09	c4b_3963299	167.03						
	c7a_10083312	72.10	c4b_6135010	167.82						
	c7a_12857173	72.84	c4b_10703398	184.82						
	c7a_5135692	73.19	c4b_2779492	187.05						
	c7a_8408079	74.40	c4b_20834700	187.88						
	c7a_17754822	74.54	c4b_18234332	189.66						
	c7a_16816770	78.85	c4b_24014246	190.82						
	c7a_18456350	80.29	c4b_24710019	191.35						
	c7a_19377124	80.59	c4b_20416931	191.41						
	c7a_10270983	81.09	c4b_27438399	192.81						
	c7a_17908688	85.44	c4b_37334976	192.89						
	c7a_24576260	86.15	c4b_36461979	193.80						
	c7a_19325608	86.27	c4b_50378421	201.67						
	c7a_19325743	92.37	c4b_18236211	203.79						
	c7a_18456087	93.07	c4b_17345413	206.79						
	c7a_15290252	93.44	c4b_50459463	206.81						
	c7a_21524967	96.02	c4b_9156286	214.79						
	c7a_18212123	100.31	c4b_42616350	215.95						
	c7a_26511407	101.74	c4b_50398077	217.91						
	c7a_10270872	101.99	c4b_37703886	218.72						
	c7a_18260788	102.39	c4b_49285361	219.61						
	c7a_18456275	104.58	c4b_37369552	220.10						
	c7a_34961087	108.42	c5a_63231883	0.00						
	c7a_18488155	110.57	c5a_63458284	30.35						
	c7a_10271008	111.13	c5a_58736074	30.63						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c7a_19315381	111.83	c5a_59020999	31.36						
	c7a_8936543	112.65	c5a_42770956	32.40						
	c7a_16773064	112.70	c5a_61728698	33.16						
	c7a_43215265	115.34	c5a_62085571	34.56						
	c7a_25011236	118.76	c5a_63551458	34.90						
	c7a_53402308	126.68	c5a_12731790	35.18						
	c7a_16945698	127.28	c5a_61313221	37.92						
	c7a_51627266	134.34	c5a_42465267	39.29						
	c7a_14565477	161.38	c5a_16228840	41.18						
	c7b_1897740	25.68	c5a_61693840	45.28						
	c7b_4808097	27.01	c5a_43234145	45.76						
	c7b_15640234	30.54	c5a_63480485	46.21						
	c7b_12052754	31.14	c5a_61387546	46.47						
	c7b_314090	32.17	c5a_61566415	46.90						
	c7b_17408929	32.25	c5a_9378516	46.93						
	c7b_5370365	32.27	c5a_61485131	48.23						
	c7b_1980230	33.62	c5a_61825563	49.44						
	c7b_1401568	34.24	c5a_61751442	50.03						
	c7b_2094164	34.72	c5a_61485247	50.18						
	c7b_15640188	34.79	c5a_37867845	50.83						
	c7b_14115861	36.70	c5a_62103865	51.04						
	c7b_12040606	36.78	c5a_62097509	51.53						
	c7b_8079929	39.00	c5a_61642294	52.48						
	c7b_16616678	39.10	c5a_49338870	52.52						
	c7b_345287	40.99	c5a_5681737	53.16						
	c7b_8080482	41.78	c5a_54750865	53.35						
	c7b_17250208	43.39	c5a_20232263	53.59						
	c7b_13245623	43.88	c5a_56011681	70.73						
	c7b_4032798	44.97	c5a_3006469	71.38						
	c7b_11268032	45.30	c5a_58487539	72.53						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c7b_15591860	45.30	c5a_28966828	72.80						
	c7b_4065988	46.66	c5a_60264051	73.55						
	c7b_987527	46.79	c5a_54096316	74.17						
	c7b_17213104	47.95	c5a_51588166	74.30						
	c7b_18646766	48.72	c5a_59477569	74.36						
	c7b_9248041	49.81	c5a_50629389	74.76						
	c7b_1657299	49.83	c5a_59866915	75.16						
	c7b_17216669	50.92	c5a_53812049	85.44						
	c7b_17551578	50.95	c5a_50647912	86.29						
	c7b_17216615	53.50	c5a_51404312	86.71						
	c7b_5178304	53.82	c5a_52037699	87.20						
	c7b_271031	54.50	c5a_2415611	88.06						
	c7b_21313605	55.52	c5a_59764175	95.34						
	c7b_20514851	55.94	c5a_2715754	95.89						
	c7b_21833986	65.79	c5a_51601576	99.31						
	c7b_46326742	74.44	c5a_44220123	99.53						
	c7b_42684427	75.80	c5a_2396322	99.95						
	c7b_29490371	78.99	c5a_54679529	102.11						
	c7b_23221308	79.34	c5a_44220483	102.26						
	c7b_29493451	84.14	c5a_42143681	102.41						
	c7b_43155675	84.61	c5a_44220053	103.65						
	c7b_43593772	88.58	c5a_53921437	105.30						
	c7b_43135498	90.33	c5a_44216434	106.64						
	c7b_22308955	90.83	c5a_45936852	107.95						
	c7b_29496177	90.89	c5a_43729415	109.40						
	c7b_29465905	91.05	c5a_43680378	111.02						
	c7b_46238235	92.99	c5a_43823742	111.98						
	c7b_29541119	94.35	c5a_44549807	113.42						
	c7b_48751067	94.68	c5a_1948795	114.32						
	c7b_46247269	96.03	c5a_26098597	114.80						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c7b_42751101	96.37	c5a_13299442	116.15						
	c7b_7746171	97.03	c5a_53921374	116.39						
	c8a_16115017	71.82	c5a_28964748	117.30						
	c8a_17289323	73.20	c5a_44548666	118.30						
	c8a_16114611	79.18	c5a_13861701	120.37						
	c8a_26299344	80.14	c5a_43175327	121.68						
	c8a_42217865	80.23	c5a_25098072	126.50						
	c8a_43042388	82.39	c5a_13857923	127.04						
	c8a_43239288	82.52	c5a_3561939	127.27						
	c8a_16829097	82.62	c5a_37625888	127.50						
	c8a_44098371	82.88	c5a_39246673	129.25						
	c8a_18503891	85.08	c5a_37478203	129.94						
	c8a_34468326	86.63	c5a_13819003	131.59						
	c8a_14434016	86.64	c5a_43680436	131.83						
	c8a_28738729	91.98	c5a_26611568	132.90						
	c8a_43989688	93.14	c5a_2026388	134.05						
	c8a_9025298	93.67	c5a_29424163	137.12						
	c8a_28227980	94.50	c5a_3352157	137.57						
	c8a_9025340	95.75	c5a_15042939	140.54						
	c8a_28739046	96.47	c5a_9165315	140.66						
	c8a_8708514	98.56	c5a_2601216	143.01						
	c8a_28722695	98.98	c5a_15395958	144.03						
	c8a_6204660	109.57	c5a_38901927	147.70						
	c8a_43234529	111.43	c5a_12328356	148.18						
	c8a_2726432	113.25	c5a_15043480	148.91						
	c8a_4986324	114.28	c5a_2016908	152.45						
	c8a_6094425	116.72	c5a_10561141	152.67						
	c8a_5123586	120.00	c5a_15681770	154.12						
	c8a_6306185	121.57	c5a_749728	154.67						
	c8b_45478377	90.29	c5a_6139446	155.76						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c8b_51004735	91.49	c5a_8191869	155.84						
	c8b_32652408	91.86	c5a_16084879	157.65						
	c8b_52787795	91.91	c5a_12437493	159.40						
	c8b_47844096	95.96	c5a_3615652	160.04						
	c8b_49744419	96.15	c5a_8191879	161.56						
	c8b_48239385	123.01	c5a_12159515	163.32						
	c8b_52848743	124.46	c5a_12337994	163.40						
	c9a_2346213	24.22	c5a_6243500	166.40						
	c9a_10207725	27.55	c5a_9150841	167.56						
	c9a_6860322	45.72	c5a_15120636	168.10						
	c9a_6789438	45.80	c5a_3505220	171.05						
	c9a_7497842	46.13	c5a_3662227	171.48						
	c9a_9017584	46.78	c5a_6139848	173.02						
	c9a_6754446	48.10	c5a_3277815	177.82						
	c9a_8590428	48.29	c5a_3598780	178.78						
	c9a_5206414	48.94	c5a_6136208	179.86						
	c9a_14129523	50.03	c5a_10674591	180.19						
	c9a_5211098	51.18	c5a_3258606	186.30						
	c9a_5208321	52.07	c5a_6242684	187.65						
	c9a_5205875	52.96	c5a_35774567	187.79						
	c9a_5643782	53.13	c5a_3599106	191.30						
	c9a_11331673	53.95	c5a_5588147	191.50						
	c9a_8985256	54.05	c5a_5354752	193.79						
	c9a_3950445	55.71	c5a_3299888	198.33						
	c9a_8970517	56.43	c5a_3642944	203.32						
	c9a_5730125	61.87	c5a_2683971	216.34						
	c9a_8985192	62.84	c5a_3234109	216.72						
	c9a_19169293	62.95	c5b_45557510	11.64						
	c9a_21332674	63.42	c5b_328117	12.39						
	c9a_12389517	63.54	c5b_659419	14.27						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9a_25914477	64.89	c5b_3515244	19.35						
	c9a_85527117	66.57	c5b_12437247	19.85						
	c9a_4131332	67.51	c5b_981486	20.04						
	c9a_4869069	67.88	c5b_28806521	20.76						
	c9a_30590969	68.24	c5b_4731274	23.71						
	c9a_14421575	69.32	c5b_1507378	24.34						
	c9a_84700578	69.83	c5b_38422605	25.14						
	c9a_85560825	70.83	c5b_658965	25.41						
	c9a_12590238	71.69	c5b_4276920	27.92						
	c9a_19631472	72.53	c5b_1081176	30.69						
	c9a_19812283	73.11	c5b_659708	30.92						
	c9a_82853723	73.78	c5b_4005688	31.36						
	c9a_25910319	74.07	c5b_9787885	32.79						
	c9a_1448252	75.75	c5b_49147428	33.73						
	c9a_84541172	76.41	c5b_3145501	35.38						
	c9a_21335113	76.42	c5b_7429373	36.50						
	c9a_975837	76.80	c5b_10483701	56.84						
	c9a_25509970	78.45	c5b_16163812	73.42						
	c9a_84298315	78.74	c5b_25240689	76.50						
	c9a_10102127	79.17	c5b_64144720	77.07						
	c9a_19283441	80.41	c5b_20667870	77.10						
	c9a_81286100	81.41	c5b_20625690	77.28						
	c9a_79992653	81.46	c5b_14974595	77.60						
	c9a_12474599	81.99	c5b_75563859	78.14						
	c9a_26213112	82.20	c5b_43473294	81.25						
	c9a_19175466	82.25	c5b_15461474	81.93						
	c9a_81537927	82.64	c5b_46256296	82.31						
	c9a_26404542	84.39	c5b_75470503	82.61						
	c9a_14862242	84.98	c5b_32327026	85.14						
	c9a_86216739	86.02	c5b_25243372	86.83						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9a_12474590	86.86	c5b_75588535	87.07						
	c9a_52832912	86.89	c5b_9651738	87.52						
	c9a_25472781	87.20	c5b_17139954	88.11						
	c9a_53050337	88.35	c5b_37718537	89.65						
	c9a_83295024	88.43	c5b_75824245	90.33						
	c9a_30155804	89.76	c5b_7070318	92.51						
	c9a_79321670	90.25	c5b_42687892	99.77						
	c9a_60700752	91.52	c5b_71907045	100.11						
	c9a_79785214	91.64	c5b_36164086	100.69						
	c9a_65657482	91.76	c5b_28277603	101.88						
	c9a_10102014	92.93	c5b_69941655	102.96						
	c9a_84435138	93.04	c5b_71906761	103.29						
	c9a_73034185	93.60	c5b_75594176	104.33						
	c9a_28832290	93.83	c5b_38777892	104.42						
	c9a_30538415	93.84	c5b_39064436	105.65						
	c9a_80032813	93.98	c5b_37939675	108.09						
	c9a_49000623	94.25	c5b_35704498	111.33						
	c9a_44326461	94.98	c5b_61248641	115.35						
	c9a_26188318	95.38	c5b_66642543	116.76						
	c9a_15428139	95.66	c5b_32499759	117.55						
	c9a_79854990	96.05	c5b_33565607	117.79						
	c9a_81222641	97.11	c5b_33086860	119.28						
	c9a_25945443	97.55	c5b_33744017	121.45						
	c9a_20505302	98.13	c5b_32757500	121.52						
	c9a_52806924	98.31	c5b_33743980	122.86						
	c9a_40538003	98.60	c5b_33758412	124.00						
	c9a_80029551	98.69	c5b_58863780	127.73						
	c9a_38466367	98.71	c5b_61247252	128.96						
	c9a_38466146	98.91	c5b_33530082	129.05						
	c9a_26419203	99.90	c5b_33337999	140.44						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9a_78621342	100.41	c5b_70696124	142.64						
	c9a_72381717	101.76	c5b_57940602	143.08						
	c9a_12007343	102.20	c5b_71658350	147.18						
	c9a_44335296	102.60	c5b_70614622	151.48						
	c9a_58276615	102.92	c5b_71098491	152.28						
	c9a_48846619	103.56	c5b_74319947	176.25						
	c9a_74150150	103.99	c5b_71892178	178.86						
	c9a_83920432	104.63	c5b_75563973	181.68						
	c9a_44327006	104.99	c5b_71817649	182.45						
	c9a_53017202	105.45	c5b_71958492	183.70						
	c9a_55109001	106.54	c5b_71957856	186.37						
	c9a_82700072	106.63	c5b_71967588	188.89						
	c9a_53052295	106.75	c5b_75687981	190.18						
	c9a_18063977	106.84	c5b_75895635	192.60						
	c9a_49000522	107.79	c5b_75210591	194.69						
	c9a_76105621	107.80	c6a_1078775	0.00						
	c9a_81895607	107.94	c6a_37764430	1.20						
	c9a_74273375	108.90	c6a_3108491	7.65						
	c9a_45615254	109.04	c6a_138204	12.61						
	c9a_11329130	109.28	c6a_9309784	15.81						
	c9a_70694846	109.38	c6a_9500179	37.37						
	c9a_59200876	115.29	c6a_2813677	37.98						
	c9a_73516686	115.45	c6a_706746	40.82						
	c9a_52796581	115.49	c6a_36565897	41.17						
	c9a_68212612	117.99	c6a_5662065	41.39						
	c9a_35702438	122.92	c6a_6458505	43.18						
	c9a_73869601	122.93	c6a_7364923	43.52						
	c9a_70493643	123.55	c6a_36890001	46.01						
	c9a_73870385	126.72	c6a_11352236	46.65						
	c9a_68212392	127.20	c6a_2843141	46.85						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9a_86484861	127.28	c6a_46240861	65.99						
	c9a_84696882	127.53	c6a_49715389	66.11						
	c9a_40537574	128.62	c6a_6703915	66.49						
	c9a_69357104	128.75	c6a_12590385	67.69						
	c9a_71258972	129.02	c6a_6497876	68.00						
	c9a_68176912	130.50	c6a_46239218	70.42						
	c9a_68198584	131.17	c6a_12421121	70.95						
	c9a_83993895	131.50	c6a_7400802	71.02						
	c9a_66999512	132.32	c6a_51609924	71.78						
	c9a_71569600	132.39	c6a_2815670	72.22						
	c9a_68764994	132.58	c6a_52042867	72.44						
	c9a_68212521	135.60	c6a_6941242	73.21						
	c9a_63765258	136.14	c6a_46239275	73.80						
	c9a_25945174	136.64	c6a_7400789	74.69						
	c9a_70493871	139.23	c6a_7677368	75.10						
	c9a_68177539	139.89	c6a_9133535	75.36						
	c9a_86388591	140.03	c6a_51609574	76.37						
	c9a_65400733	140.53	c6a_12386927	76.81						
	c9a_72988227	140.59	c6a_44353629	77.06						
	c9a_69083915	141.23	c6a_8051041	79.72						
	c9a_72974345	141.53	c6a_49288913	80.46						
	c9a_79175438	143.14	c6a_31941374	80.50						
	c9a_83905467	143.54	c6a_11661214	80.98						
	c9a_60596498	143.97	c6a_49288868	84.17						
	c9a_70478520	144.16	c6a_29664369	84.76						
	c9a_63327221	145.18	c6a_45731581	87.89						
	c9a_65150187	146.62	c6a_41895375	91.00						
	c9a_86054146	147.43	c6a_44927383	91.17						
	c9a_86386603	149.66	c6a_44731149	91.50						
	c9a_60235412	149.69	c6a_13394134	92.51						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9a_72040797	150.72	c6a_37746548	92.84						
	c9a_83810701	151.72	c6a_48207185	93.17						
	c9a_61360975	152.08	c6a_7458078	94.25						
	c9a_70511060	152.25	c6a_44578152	95.20						
	c9a_68339707	154.62	c6a_44043545	95.26						
	c9a_81465537	155.33	c6a_41942900	97.38						
	c9a_61318841	155.76	c6a_44756111	97.83						
	c9a_83563370	155.82	c6a_44756115	98.11						
	c9a_85396592	159.26	c6a_49589875	98.93						
	c9a_80102840	160.36	c6a_44582668	100.36						
	c9a_82005780	161.46	c6a_48207236	101.52						
	c9a_26327952	174.45	c6a_52556620	101.55						
	c9b_375454	29.84	c6a_45647558	103.84						
	c9b_1876068	30.09	c6a_44453238	106.20						
	c9b_13462	30.40	c6a_45591681	109.26						
	c9b_57640593	30.62	c6a_43381822	114.38						
	c9b_18092073	44.36	c6a_51989508	116.39						
	c9b_4034489	44.40	c6a_49193398	118.12						
	c9b_9126947	45.60	c6a_49346677	120.67						
	c9b_8749991	46.07	c6b_701219	11.89						
	c9b_5349174	46.65	c6b_119963	15.93						
	c9b_6473871	46.85	c6b_687575	16.89						
	c9b_27395917	47.07	c6b_2883272	21.49						
	c9b_6433764	47.57	c6b_2971897	22.28						
	c9b_7410330	49.85	c6b_695151	22.88						
	c9b_18677791	49.87	c6b_700858	25.99						
	c9b_1761275	51.75	c6b_2814093	28.02						
	c9b_1153323	51.79	c6b_3229056	32.72						
	c9b_8934781	52.05	c6b_2785306	34.43						
	c9b_10432314	52.78	c6b_3211574	34.46						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9b_976983	52.83	c6b_3448192	36.82						
	c9b_3933983	53.11	c6b_3211697	41.10						
	c9b_54372286	54.00	c6b_8375347	42.10						
	c9b_6508936	54.40	c6b_27053215	45.77						
	c9b_7325726	55.37	c6b_3561332	46.08						
	c9b_1997271	58.13	c6b_3764436	48.04						
	c9b_48887331	58.19	c6b_8409092	50.06						
	c9b_7325700	58.27	c6b_17870443	50.60						
	c9b_7191347	58.49	c6b_3561225	51.50						
	c9b_18714843	59.29	c6b_8783275	54.07						
	c9b_14918182	60.04	c6b_8411661	56.32						
	c9b_4456842	60.37	c6b_26218587	57.67						
	c9b_5220945	60.91	c6b_3561428	60.34						
	c9b_7918908	61.65	c6b_7007162	60.76						
	c9b_9585221	62.29	c6b_19647413	73.17						
	c9b_4350020	62.77	c6b_39814816	73.73						
	c9b_9585213	62.84	c6b_26378202	74.48						
	c9b_507643	62.96	c6b_19544128	77.79						
	c9b_2967095	76.62	c6b_22221244	79.42						
	c9b_888160	76.68	c6b_38155202	85.19						
	c9b_16954817	77.92	c6b_32058921	85.87						
	c9b_57490119	86.94	c6b_28116840	87.07						
	c9b_40116329	87.06	c6b_44335370	87.47						
	c9b_16677878	88.19	c6b_55471465	91.62						
	c9b_62678786	95.29	c6b_45784842	92.58						
	c9b_58239650	95.59	c6b_44204750	92.70						
	c9b_18568967	96.70	c6b_32074943	95.99						
	c9b_58157163	97.05	c6b_42602547	96.69						
	c9b_20167230	97.34	c6b_44204435	100.42						
	c9b_24346031	97.35	c6b_26218370	100.63						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Single Marker Analysis	c9b_58251251	100.71	c6b_47698023	103.70						
	c9b_62855605	100.93	c6b_49045374	104.61						
	c9b_20154932	100.97	c6b_43462025	105.16						
	c9b_67623918	100.99	c6b_42410977	105.41						
	c9b_22348365	113.38	c6b_47754837	106.76						
	c9b_67576749	113.43	c6b_49099646	107.47						
	c9b_67484962	115.46	c6b_49045283	109.05						
	c9b_57858094	115.96	c6b_47842743	109.16						
	c9b_29521058	116.20	c6b_54495712	144.89						
	c9b_68512574	117.63	c6b_48903756	146.54						
	c9b_10914916	117.97	c6b_54321443	153.88						
	c9b_37380674	129.07	c7a_47604099	0.00						
	c9b_37243647	129.53	c7a_3893719	36.37						
	c9b_35083929	131.09	c7a_2975351	38.63						
	c9b_68580569	131.39	c7a_5653737	39.98						
	c9b_48228422	131.78	c7a_5972159	40.03						
	c9b_48107467	132.30	c7a_11741385	40.49						
	c9b_8351032	132.92	c7a_24976636	40.79						
	c9b_69563897	133.55	c7a_4947451	41.33						
	c9b_62970588	134.00	c7a_4947473	42.83						
	c9b_69563158	134.17	c7a_5357467	43.13						
	c9b_42207491	151.57	c7a_8958662	43.16						
	c9b_35771707	151.69	c7a_9226796	44.20						
	c9b_68396079	151.82	c7a_4372034	44.36						
	c9b_65378892	151.87	c7a_5580251	46.33						
	c9b_43190069	151.90	c7a_2258058	46.71						
			c7a_4917471	63.25						
			c7a_8758326	63.60						
			c7a_7962009	64.18						
			c7a_9103821	64.82						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c7a_22264020	65.38						
			c7a_5077821	66.20						
			c7a_12694176	66.39						
			c7a_23639836	66.81						
			c7a_5135730	67.79						
			c7a_9103785	68.04						
			c7a_8206670	68.30						
			c7a_23337859	69.42						
			c7a_6956581	69.93						
			c7a_8422837	70.66						
			c7a_10814898	71.09						
			c7a_10083312	72.10						
			c7a_19377124	80.59						
			c7a_10270983	81.09						
			c7a_18212222	89.11						
			c7a_17032976	89.18						
			c7a_19325645	89.68						
			c7a_9330204	91.53						
			c7a_19325743	92.37						
			c7a_18456087	93.07						
			c7a_15290252	93.44						
			c7a_18144214	93.88						
			c7a_21524967	96.02						
			c7a_10083158	96.46						
			c7a_18575151	97.39						
			c7a_18260836	98.53						
			c7a_18212119	98.57						
			c7a_18212123	100.31						
			c7a_26511407	101.74						
			c7a_10270872	101.99						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c7a_18260788	102.39						
			c7a_21842947	103.66						
			c7a_18456275	104.58						
			c7a_34961087	108.42						
			c7a_18488155	110.57						
			c7a_10271008	111.13						
			c7a_19315381	111.83						
			c7a_8936543	112.65						
			c7a_16773064	112.70						
			c7a_43215265	115.34						
			c7a_25011236	118.76						
			c7a_21864078	120.34						
			c7a_40679085	121.16						
			c7a_14141194	126.05						
			c7a_53402308	126.68						
			c7a_16945698	127.28						
			c7a_51627266	134.34						
			c7a_5007502	144.61						
			c7a_14565477	161.38						
			c7b_4808097	27.01						
			c7b_15640234	30.54						
			c7b_12052754	31.14						
			c7b_314090	32.17						
			c7b_17408929	32.25						
			c7b_5370365	32.27						
			c7b_1980230	33.62						
			c7b_1401568	34.24						
			c7b_2094164	34.72						
			c7b_15640188	34.79						
			c7b_8079929	39.00						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c7b_16616678	39.10						
			c7b_345287	40.99						
			c7b_8080482	41.78						
			c7b_17250208	43.39						
			c7b_13245623	43.88						
			c7b_4032798	44.97						
			c7b_11268032	45.30						
			c7b_15591860	45.30						
			c7b_4065988	46.66						
			c7b_987527	46.79						
			c7b_17213104	47.95						
			c7b_18646766	48.72						
			c7b_9248041	49.81						
			c7b_1657299	49.83						
			c7b_17216669	50.92						
			c7b_17551578	50.95						
			c7b_17216615	53.50						
			c7b_5178304	53.82						
			c7b_271031	54.50						
			c7b_21313605	55.52						
			c7b_20514851	55.94						
			c7b_9056525	58.75						
			c7b_21284425	59.38						
			c7b_13406289	60.69						
			c7b_14328568	61.09						
			c7b_20588371	61.34						
			c7b_21663268	64.96						
			c7b_21833986	65.79						
			c7b_21480820	65.91						
			c7b_42684427	75.80						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c7b_29490371	78.99						
			c7b_23221308	79.34						
			c7b_43099416	82.46						
			c7b_29493451	84.14						
			c7b_43155675	84.61						
			c7b_43593772	88.58						
			c7b_43135498	90.33						
			c7b_22308955	90.83						
			c7b_29496177	90.89						
			c7b_29465905	91.05						
			c7b_46238235	92.99						
			c7b_29541119	94.35						
			c7b_48751067	94.68						
			c7b_46247269	96.03						
			c7b_42751101	96.37						
			c7b_7746171	97.03						
			c8a_16114611	79.18						
			c8a_26299344	80.14						
			c8a_42217865	80.23						
			c8a_43042388	82.39						
			c8a_43239288	82.52						
			c8a_16829097	82.62						
			c8a_44098371	82.88						
			c8a_18503891	85.08						
			c8a_34468326	86.63						
			c8a_14434016	86.64						
			c8a_28738729	91.98						
			c8a_43989688	93.14						
			c8a_9025298	93.67						
			c8a_28227980	94.50						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c8a_9025340	95.75						
			c8a_28739046	96.47						
			c8a_8708514	98.56						
			c8a_28722695	98.98						
			c8a_1620362	106.71						
			c8a_36958774	108.27						
			c8a_6204660	109.57						
			c8a_43234529	111.43						
			c8a_2726432	113.25						
			c8a_4986324	114.28						
			c8a_6094425	116.72						
			c8a_5123586	120.00						
			c8a_6306185	121.57						
			c8b_11824406	33.73						
			c8b_16857858	36.30						
			c8b_8356787	37.83						
			c8b_38883109	49.22						
			c8b_30389102	50.26						
			c8b_42728157	52.10						
			c8b_38883009	54.57						
			c8b_15961784	54.79						
			c8b_17746322	55.36						
			c8b_30389740	58.35						
			c8b_30386260	58.43						
			c8b_36380823	59.89						
			c8b_42669969	61.17						
			c8b_38883062	61.97						
			c8b_31539943	62.53						
			c8b_15958634	63.02						
			c8b_36381444	63.18						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c8b_20531043	63.59						
			c8b_6997339	64.29						
			c8b_28979657	65.37						
			c8b_27077874	86.31						
			c8b_53663206	86.34						
			c8b_45271748	86.64						
			c8b_52402981	88.08						
			c8b_47427019	88.90						
			c8b_48412220	89.15						
			c8b_45478377	90.29						
			c8b_51004735	91.49						
			c8b_32652408	91.86						
			c8b_52787795	91.91						
			c8b_52862198	93.72						
			c8b_47844096	95.96						
			c8b_49744419	96.15						
			c8b_48425907	97.29						
			c8b_52861737	97.89						
			c8b_2933221	98.10						
			c8b_48239385	123.01						
			c8b_52848743	124.46						
			c9a_2346213	24.22						
			c9a_1026754	25.99						
			c9a_1068597	27.53						
			c9a_10207725	27.55						
			c9a_8436527	27.55						
			c9a_853405	27.88						
			c9a_942162	31.53						
			c9a_1916065	31.95						
			c9a_6860322	45.72						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9a_6789438	45.80						
			c9a_7497842	46.13						
			c9a_9017584	46.78						
			c9a_6754446	48.10						
			c9a_8590428	48.29						
			c9a_5206414	48.94						
			c9a_14129523	50.03						
			c9a_5211098	51.18						
			c9a_5208321	52.07						
			c9a_5205875	52.96						
			c9a_5643782	53.13						
			c9a_11331673	53.95						
			c9a_8985256	54.05						
			c9a_5750736	54.55						
			c9a_8708523	54.74						
			c9a_3950445	55.71						
			c9a_8970517	56.43						
			c9a_14505855	57.34						
			c9a_5361245	57.63						
			c9a_2746468	58.74						
			c9a_8437777	58.94						
			c9a_5750686	59.10						
			c9a_13815130	59.67						
			c9a_5730125	61.87						
			c9a_8985192	62.84						
			c9a_19169293	62.95						
			c9a_21332674	63.42						
			c9a_12389517	63.54						
			c9a_25914477	64.89						
			c9a_85527117	66.57						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9a_4131332	67.51						
			c9a_4869069	67.88						
			c9a_30590969	68.24						
			c9a_14421575	69.32						
			c9a_84700578	69.83						
			c9a_85560825	70.83						
			c9a_82853723	73.78						
			c9a_25910319	74.07						
			c9a_1448252	75.75						
			c9a_84541172	76.41						
			c9a_21335113	76.42						
			c9a_975837	76.80						
			c9a_25509970	78.45						
			c9a_84298315	78.74						
			c9a_10102127	79.17						
			c9a_19283441	80.41						
			c9a_81286100	81.41						
			c9a_79992653	81.46						
			c9a_12474599	81.99						
			c9a_26213112	82.20						
			c9a_19175466	82.25						
			c9a_81537927	82.64						
			c9a_26404542	84.39						
			c9a_14862242	84.98						
			c9a_86216739	86.02						
			c9a_53050337	88.35						
			c9a_83295024	88.43						
			c9a_30155804	89.76						
			c9a_79321670	90.25						
			c9a_60700752	91.52						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9a_79785214	91.64						
			c9a_65657482	91.76						
			c9a_10102014	92.93						
			c9a_84435138	93.04						
			c9a_73034185	93.60						
			c9a_28832290	93.83						
			c9a_30538415	93.84						
			c9a_80032813	93.98						
			c9a_49000623	94.25						
			c9a_44326461	94.98						
			c9a_26188318	95.38						
			c9a_79854990	96.05						
			c9a_81222641	97.11						
			c9a_25945443	97.55						
			c9a_20505302	98.13						
			c9a_52806924	98.31						
			c9a_40538003	98.60						
			c9a_80029551	98.69						
			c9a_38466367	98.71						
			c9a_38466146	98.91						
			c9a_26419203	99.90						
			c9a_78621342	100.41						
			c9a_72381717	101.76						
			c9a_12007343	102.20						
			c9a_44335296	102.60						
			c9a_58276615	102.92						
			c9a_48846619	103.56						
			c9a_74150150	103.99						
			c9a_83920432	104.63						
			c9a_44327006	104.99						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9a_53017202	105.45						
			c9a_82700072	106.63						
			c9a_53052295	106.75						
			c9a_18063977	106.84						
			c9a_49000522	107.79						
			c9a_76105621	107.80						
			c9a_81895607	107.94						
			c9a_74273375	108.90						
			c9a_40537574	128.62						
			c9a_69357104	128.75						
			c9a_71258972	129.02						
			c9a_68176912	130.50						
			c9a_68198584	131.17						
			c9a_83993895	131.50						
			c9a_66999512	132.32						
			c9a_71569600	132.39						
			c9a_68764994	132.58						
			c9a_68212521	135.60						
			c9a_63765258	136.14						
			c9a_25945174	136.64						
			c9a_70493871	139.23						
			c9a_86388591	140.03						
			c9a_65400733	140.53						
			c9a_72988227	140.59						
			c9a_69083915	141.23						
			c9a_79175438	143.14						
			c9a_83905467	143.54						
			c9a_60596498	143.97						
			c9a_70478520	144.16						
			c9a_63327221	145.18						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9a_65150187	146.62						
			c9a_86054146	147.43						
			c9a_86386603	149.66						
			c9a_60235412	149.69						
			c9a_72040797	150.72						
			c9a_61360975	152.08						
			c9a_70511060	152.25						
			c9a_68339707	154.62						
			c9a_81465537	155.33						
			c9a_61318841	155.76						
			c9a_83563370	155.82						
			c9a_85396592	159.26						
			c9a_82005780	161.46						
			c9a_26327952	174.45						
			c9b_58012802	14.05						
			c9b_6468904	16.43						
			c9b_851588	19.54						
			c9b_58315654	20.24						
			c9b_6525944	21.23						
			c9b_1673031	22.37						
			c9b_6469352	22.94						
			c9b_57494040	24.40						
			c9b_1027425	24.87						
			c9b_562625	25.43						
			c9b_728769	25.64						
			c9b_1883319	27.11						
			c9b_375454	29.84						
			c9b_1876068	30.09						
			c9b_13462	30.40						
			c9b_57640593	30.62						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9b_6494213	32.07						
			c9b_7487301	43.33						
			c9b_18092073	44.36						
			c9b_4034489	44.40						
			c9b_9126947	45.60						
			c9b_1818678	46.00						
			c9b_1123538	46.00						
			c9b_8749991	46.07						
			c9b_5349174	46.65						
			c9b_6473871	46.85						
			c9b_27395917	47.07						
			c9b_6433764	47.57						
			c9b_7410330	49.85						
			c9b_18677791	49.87						
			c9b_1761275	51.75						
			c9b_1153323	51.79						
			c9b_8934781	52.05						
			c9b_10432314	52.78						
			c9b_976983	52.83						
			c9b_3933983	53.11						
			c9b_54372286	54.00						
			c9b_6508936	54.40						
			c9b_7325726	55.37						
			c9b_48887331	58.19						
			c9b_7325700	58.27						
			c9b_7191347	58.49						
			c9b_18714843	59.29						
			c9b_14918182	60.04						
			c9b_4456842	60.37						
			c9b_5220945	60.91						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9b_7918908	61.65						
			c9b_9585221	62.29						
			c9b_4350020	62.77						
			c9b_9585213	62.84						
			c9b_507643	62.96						
			c9b_13663836	63.10						
			c9b_2894331	63.47						
			c9b_11671351	64.02						
			c9b_48887401	64.18						
			c9b_4310217	65.22						
			c9b_12085007	65.61						
			c9b_55037092	66.04						
			c9b_14917855	67.00						
			c9b_3933949	69.17						
			c9b_21135362	69.39						
			c9b_12103625	70.06						
			c9b_5178172	71.00						
			c9b_14600010	71.70						
			c9b_31388569	72.33						
			c9b_10551543	72.93						
			c9b_14216580	73.33						
			c9b_52957960	73.91						
			c9b_2855292	73.92						
			c9b_13882267	74.25						
			c9b_10871528	74.73						
			c9b_8029410	75.45						
			c9b_2967095	76.62						
			c9b_888160	76.68						
			c9b_13701731	77.47						
			c9b_16954817	77.92						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9b_13701739	78.94						
			c9b_52921113	78.96						
			c9b_20098326	81.49						
			c9b_57982444	81.87						
			c9b_16900989	82.78						
			c9b_59077032	83.31						
			c9b_18374629	83.84						
			c9b_12081495	85.79						
			c9b_57490119	86.94						
			c9b_40116329	87.06						
			c9b_16677878	88.19						
			c9b_19374988	89.08						
			c9b_62975072	90.10						
			c9b_62774847	90.97						
			c9b_57398724	92.33						
			c9b_8220728	92.39						
			c9b_19327850	93.70						
			c9b_20167327	93.75						
			c9b_48084769	94.76						
			c9b_5253534	94.93						
			c9b_62678786	95.29						
			c9b_58239650	95.59						
			c9b_18568967	96.70						
			c9b_58157163	97.05						
			c9b_20167230	97.34						
			c9b_24346031	97.35						
			c9b_19899430	98.92						
			c9b_58244409	99.36						
			c9b_10591716	99.64						
			c9b_21477220	100.41						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9b_58251251	100.71						
			c9b_62855605	100.93						
			c9b_20154932	100.97						
			c9b_67623918	100.99						
			c9b_40116398	102.63						
			c9b_21147283	102.63						
			c9b_64473200	106.42						
			c9b_8402300	106.52						
			c9b_67484962	115.46						
			c9b_57858094	115.96						
			c9b_29521058	116.20						
			c9b_68512574	117.63						
			c9b_10914916	117.97						
			c9b_37380674	129.07						
			c9b_37243647	129.53						
			c9b_35083929	131.09						
			c9b_68580569	131.39						
			c9b_48228422	131.78						
			c9b_48107467	132.30						
			c9b_8351032	132.92						
			c9b_69563897	133.55						
			c9b_62970588	134.00						
			c9b_69563158	134.17						
			c9b_55437573	137.92						
			c9b_69719040	138.01						
			c9b_68580721	139.49						
			c9b_67987631	139.71						
			c9b_69803057	140.99						
			c9b_65281350	144.77						
			c9b_69523359	145.36						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
			c9b_54715977	145.52						
			c9b_42207491	151.57						
			c9b_35771707	151.69						
			c9b_68396079	151.82						
			c9b_65378892	151.87						
			c9b_43190069	151.90						
			c9b_55434769	152.05						
			c9b_69654442	152.62						
			c9b_61410379	152.79						
Interval Mapping (IM)	c1a_70541448	12.62	c1a_71531924	9.31	c9a_30155804	89.76				
	c2b_72315353	65.18	c1b_8290479	48.51	c9a_53017202	105.45				
	c2b_61963807	93.92	c2a_13791205	146.64						
	c2b_65275379	108.07	c2a_77652148	154.64						
	c3a_48763659	28.19	c2a_77470461	194.66						
	c3b_17624128	58.03	c2a_76843098	201.69						
	c4a_1909672	25.26	c2b_72315353	65.18						
	c4a_4417407	31.17	c2b_66683204	98.11						
	c4b_40512089	63.66	c2b_65275379	108.07						
	c5a_26098597	114.80	c2b_32150332	159.27						
	c5a_13857923	127.04	c2b_21851537	180.06						
	c5a_2026388	134.05	c2b_14679039	188.01						
	c5a_8191869	155.84	c3a_18077235	114.74						
	c5a_8191879	161.56	c3b_17624128	58.03						
	c5a_15120636	168.10	c4a_1909672	25.26						
	c5a_3277815	177.82	c4a_4417407	31.17						
	c5a_3642944	203.32	c4a_18121450	81.66						
	c5b_28806521	20.76	c4a_44248251	92.69						
	c6b_3764436	48.04	c4b_40512089	63.66						
	c7a_5007502	144.61	c4b_920120	149.83						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Interval Mapping (IM)	c7b_5370365	32.27	c5a_63231883	0.00						
	c7b_345287	40.99	c5a_62085571	34.56						
	c7b_1657299	49.83	c5a_9378516	46.93						
	c7b_43155675	84.61	c5a_2396322	99.95						
	c8a_18503891	85.08	c5a_26098597	114.80						
	c9a_84700578	69.83	c5a_25098072	126.50						
	c9a_84298315	78.74	c5a_2026388	134.05						
	c9a_26404542	84.39	c5a_16084879	157.65						
	c9a_84435138	93.04	c5a_15120636	168.10						
	c9a_53017202	105.45	c5a_10674591	180.19						
	c9a_60596498	143.97	c5a_3642944	203.32						
	c9b_18677791	49.87	c5b_981486	20.04						
	c9b_5220945	60.91	c5b_20667870	77.10						
	c9b_8351032	132.92	c5b_32327026	85.14						
			c5b_28277603	101.88						
			c5b_71967588	188.89						
			c6a_41895375	91.00						
			c6a_44756111	97.83						
			c6b_695151	22.88						
			c6b_3764436	48.04						
			c6b_47698023	103.70						
			c6b_48903756	146.54						
			c7a_5135730	67.79						
			c7a_43215265	115.34						
			c7a_53402308	126.68						
			c7a_5007502	144.61						
			c7b_345287	40.99						
			c7b_1657299	49.83						
			c7b_43155675	84.61						
			c8a_18503891	85.08						

Table 3B. (continued).

Analysis	Biomass Yield		Biomass Yield-Ardmore		Biomass Yield-Knoxville		Seed Weight		Germination	
	Marker	Position	Marker	Position	Marker	Position	Marker	Position	Marker	Position
Interval Mapping (IM)			c9a_5208321	52.07						
			c9a_5730125	61.87						
			c9a_4869069	67.88						
			c9a_84435138	93.04						
			c9b_18677791	49.87						
			c9b_5220945	60.91						
			c9b_888160	76.68						
			c9b_58157163	97.05						
			c9b_48228422	131.78						

CONCLUSIONS

Switchgrass is a warm-season C4 grass with perennial growth habit that is used for forage, soil conservation, and biofuel production. Prior cultivar breeding on switchgrass was concentrated on improvement of forage yield and nutritional quality (Vogel and Jung, 2001). Switchgrass has high biomass yield potential and the ability to sequester a large amount of atmospheric CO_2 into organic carbon (Blanco-Canqui, 2010). Due to these advantages, switchgrass was chosen as the candidate herbaceous species for the bioenergy feedstock production in the United States. This resulted in widespread research interest in enhancing its biomass yield and feedstock quality through breeding and genomics research. The primary goal of this study is biomass yield improvement for use as a bioenergy feedstock. The research plan was partitioned into three main objectives: (i) evaluate the genetic diversity among lowland switchgrass populations using microsatellite markers; (ii) assess genetic variation in the Alamo half-sib population developed through phenotypic selection; (iii) and identify QTLs and molecular markers associated with biomass yield and establishment related seed traits using a NAM (Nested Association Mapping) population.

The first research aims to understand the extent of genetic variation among and within different germplasm accessions of switchgrass that will aid to their efficient utilization in cultivar breeding. Results showed significant morphological variations ($P<0.05$) among and within germplasm accessions. Cluster analysis using phenotypic data revealed four groups, but the separation did not follow ancestral relationships. Molecular analysis revealed landraces exhibiting higher genetic variation, larger number of unique alleles, and high polymorphism while improved populations showed reduced diversity, few unique alleles, and less polymorphism. The Analysis of Molecular Variance (AMOVA) revealed that among genotypes within populations and among populations explained 84 and 16% of molecular genetic variations respectively. Our results

showed a reduced level of molecular diversity in improved cultivars which could be an issue if the improved cultivar is to be used in recurrent selection. The issue could be addressed by following among and within family selection that would ensure greater allelic diversity through the cycles of recurrent selections (Casler and Brummer, 2008). However, environmental influence on single plant performance could limit the genetic gain following AWF selection. Using at least two clonal replicates in family evaluation would allow parsing out residual variance thus improving the selection efficiency.

After confirmation of genetic diversity among desired populations based on important traits especially biomass yield, the next step would be genetic improvement through breeding efforts. The second research aims to assess genetic variation and genetic gain in a structured population (AHS) for cultivar development. Results revealed significant variation ($P<0.05$) among AHS for biomass yield, tillering ability and spring vigor suggesting the importance of additive genetic variation in these traits. Overall mean biomass yield of AHS was not different from Alamo control demonstrating the inefficiency of phenotypic selection from sward. Mean biomass yield of ACHS was 15 and 20% less compared with Alamo and AHS, respectively. However, results showed great potential for biomass yield improvement using AWF due to the large genetic variation associated with among genotypes within the family. It may be advisable to consider evaluating at least two clonal replicates of each genotype within HS to separate the environmental influence on AWF selection. Adopting regional breeding programs based on hardiness zone and well-characterized ecoregions would help address issues of genotype × environment interactions.

Although genetic improvement can be achieved through conventional breeding strategies, biomass yield and other traits are complex traits that are highly influenced by the environment. To dissect the genetic factors of these complex traits, identification of quantitative trait loci (QTL) is

important. QTL mapping can identify molecular markers linked to economically important traits and selection using these markers could accelerate crop improvement for biofuel production. Our study was able to identify several QTLs for biomass yield, seed weight, and germination and find SNPs closely linked with these QTL. These associated SNPs will be useful in marker-assisted breeding. However, these QTLs should be validated by searching for candidate genes that are related to biomass yield, seed weight, and germination using independent populations.

Although several QTL studies have been conducted for biomass yield, our research utilized a NAM population as compared to previous studies that are mostly biparental mapping populations (Lowry *et al*., 2015; Serba *et al*., 2015). We were able to detect more QTLs with greater positive additive effects as compared to previous studies, thus elucidating the advantage of using more parents in mapping population development. Also, to our knowledge, this is the first QTL study for seed weight and seed germination in switchgrass providing insights on the presence of seed weight and germination QTLs. Once validated, screening switchgrass populations for the desired trait will be possible using the associated markers that will hasten cultivar improvement in switchgrass breeding.

In summary, switchgrass is a perennial warm-season grass that has a high biomass yield potential. Improved biomass yield is an important trait for efficient biofuel production. However, biomass yield is a complex trait that is highly influenced by the environment. Combined conventional and molecular breeding efforts could hasten genetic improvement in switchgrass. The results of these studies will be useful for future breeding efforts in switchgrass and other perennial grasses.

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VITA

The author was born on the 27th of May 1983 in Los Banos, Laguna, Philippines. She is the second born daughter of Ms. Myrna Ontolan Dalid and the late Engr. James Gonzaga Dalid. She attended the University of the Philippines Los Banos (UPLB) and graduated with a Bachelor of Science degree in Biology with Genetics as her major in 2004. In the same year, she worked as a Research Analyst at the Bureau of Fisheries and Aquatic Resources (BFAR)-Fish Health Section in the Philippines. After a year, she joined the International Rice Research Institute (IRRI) where she worked for seven years.

Her thirst for knowledge led her to pursue graduate studies. While working at IRRI, she enrolled in the Master of Science in Genetics program with a minor in Molecular Biology and Biotechnology at UPLB and graduated in 2011. In 2013, the author joined the PhD program in Plant, Soil, and Environmental Science with a concentration in Plant Breeding and a minor in Statistics at the University of Tennessee, Knoxville and is expected to graduate in August 2018.

The author's main career goal is to make a difference in the world through plant breeding and genetics research that will help alleviate world hunger. The author also hopes to go back to her country one day and work as a professor in plant breeding and genetics to share the knowledge she gained from her graduate studies in the United States.